SOME RELATIONSHIPS BETWEEN PHYSIOLOGY AND STORAGE BEHAVIOUR IN INDIVIDUAL APPLES

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Except as stated herein this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and that, to the best of my knowledge and belief the thesis contains no copy or paraphrase of material previously published or written by another person except when due reference is made in the text of the thesis.

Author

The papers whose titles are given below are included at the end of this thesis as supporting material :

"The Physiology of Growth in Apple Fruits, VII Between tree variation in cell physiology in relation to disorder incidence." D. Martin, T.L. Lewis, and J. Cerny.

"Low Oxygen Gas Storage Trials of Apples in Tasmania." D. Martin and J. Cerny.

"Bitter Pit in the Apple Variety Cleopatra in Tasmania in Relation to Calcium and Magnesium." D. Martin, T.L.Lewis, and J. Cerny.

"Jonathan Spot - Three Factors Related to Incidence : Fruit Size, Breakdown, and Seed Numbers." D. Martin, T.L.Lewis and J. Cerny.

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SUMMARY

The relations between storage behaviour and some physiological characteristics of individual apples have been studied.

The fruits of a single tree have shown considerable individual variability in the different characteristics.

Improved methods have been devised for extracting samples of the internal atmosphere of apples and for measuring respiratory activity on the basis of oxygen uptake.

The investigations have shown no evidence that a higher concentration of internal carbon dioxide in the apple increases its susceptibility to breakdown.

Internal carbon dioxide was shown to be positively correlated with the respiration rate and protein nitrogen content.

The rate of oxygen uptake measured shortly after picking was positively correlated with the incidence of bitter pit in Cox variety and of Jonathan spot in Jonathan. These disorders manifested themselves after 14 or more weeks of cool storage.

The relation between Jonathan spot and fruit size in apples which were subjected to insertion of a gas pipette and to delay of ten days before cool storage was anomalous compared with uninjured fruit stored immediately.

There was a strong positive correlation between the rate of yellowing and the cell volume of the fruit.

Correlations found between certain characteristics, and differences found between fruits of light and heavy crops were in accord with findings reported by other workers.

Further investigations are now in progress which include studies of the resistances to gaseous exchange and the role of mineral elements.

INTRODUCTION

Storage disorders of apples have been studied for a long time, and their incidence has been related to many factors. Most of the work has been carried out with large samples of fruit, sometimes from several trees and perhaps from a number of different orchards. In some cases the studies have been repeated during a number of consecutive seasons.

The advantage of such work has been that any relationship discovered could be generally accepted. On the other hand, the heterogeneity within and between samples has made the finding of significant correlations very difficult. Considerable variation occurs in a given year amongst apples from a single tree, and even from a single branch. Each apple behaves more or less as an individual, and the differences between them may be so great that even a few individuals in a fairly large sample may influence the result to such an extent that the conclusion from the experimental observations may be affected. (See Table 1.)p.51

If apples from one tree are subjected to a certain period of cool storage, a number of fruits may develop a disorder. Among the affected fruits there may be a large variation in intensity and in the time taken for the disorder to manifest itself. It is often impossible to say why certain fruits have been affected while others have not.

From the large sample used for storage a small random sample is often taken soon after harvest and used for observation and chemical analysis. It is impossible to be sure that this small random sample is a representative sample in respect of disorders, since after harvest there is nothing in the appearance of the fruits to indicate which ones will develop disorders in subsequent storage.

In spite of these difficulties, Martin and co-workers (Martin 1954, Martin, Lewis and Cerny 1954) have been able to demonstrate a very close positive correlation between the incidence of the physiological disorders bitter pit and breakdown and the mean fruit weight per tree in any season, and also between fruit weight and incidence within a tree. In addition an interaction has been found between these two disorders, each tending to suppress the other. More recently they have shown (Martin, Lewis and Cerny 1961) that these principles also apply to a third physiological disorder, Jonathan spot, when it occurs alone. An interaction between Jonathan spot and breakdown has also been demonstrated.

Earlier, Martin and Lewis (1952) had shown that light crop fruits had a higher rate of respiration per unit of protein nitrogen, and also larger cells, than heavy crop fruits, which are characteristically smaller. They suggested that the large cells of light crop fruits might be unable to maintain an efficient transfer of energy for

protein maintenance at the higher rate required, and that this might explain the more rapid senescence of light crop fruits as well as their higher susceptibility to disorders.

Since it was known from the work of Kidd and West (1927), Eaves (1938), Martin and Carne (1950) and Martin and Cerny (1956) that higher than normal concentrations of carbon dioxide in the external atmosphere increase the susceptibility of apple fruits to breakdown, an alternative hypothesis was that disorder incidence might be related to difficulties of gaseous exchange, causing accumulation of carbon dioxide within the fruit tissue and thereby interfering with normal respiration. Difficulties of gaseous exchange might be associated with a smaller fruit surface : fruit volume ratio in the larger fruits characteristic of light crop trees, and in the larger fruits within a tree, and with greater cuticle thickness or smaller lenticel area : fruit volume ratio or both, according as gaseous exchange is through cuticle or lenticels or both.

The work described in this thesis was designed to test the latter hypothesis. As such work would necessarily involve observations on individual fruits it would yield information as to the variation in a number of attributes between fruits. The feasibility of using such fruits in experiments relating fruit physiology to storage behaviour

could thus be tested at the same time.

Various observations, measurements and analyses were carried out on individual fruits during the storage period, and the results were statistically treated to determine whether any characteristics of physiological behaviour or chemical composition were interrelated, or related to the incidence or severity of physiological disorders. Two varieties of apples were used for these studies, viz. Cox's Orange Pippin, which is highly susceptible to physiological disorders, especially bitter pit and breakdown, and Jonathan, which is economically one of the most important varieties grown in Tasmania. In each variety individual fruits from light and heavy crop trees were examined for physiological disorders, and their relationships to fruit size, seed number, colour, internal carbon dioxide, oxygen uptake, cell size and chemical composition were investigated, as well as any correlations between these attributes.

REVIEW OF THE LITERATURE

Workers in the post-harvest physiology of apples have directed much effort towards an understanding of the factors which affect keeping quality. It is therefore understandable that a large volume of literature exists on the subject. Even neglecting work on such factors as rootstocks, soil types, tree age, climate, etc., much study has been made of the apple fruit itself, and relations have been found between certain characteristics of fruits and their keeping quality. In the discussion of findings reported in the literature, each of these characteristics and each of the physiological disorders in question will be considered in turn.

Fruit size

A relation between fruit size within a tree and disorder incidence has been reported by Palmer (1931), by Carne and Martin (1935), and by Trout et al. (1940). Fruit size is related particularly closely to the incidence of core flush (Hoblyn 1938) and of Jonathan spot, bitter pit and deep scald (Martin 1953). Martin concludes that this may be due partly to the maturity effect (fruit left on the tree continues to grow, i.e. to increase in size), but that this could not be the case with bitter pit in Cox and Sturmer, for in these varieties pit incidence decreases with maturity.

Smith (1940) concluded that differences in size of mature apples were due to differences in both the amount of cell division and the degree of cell enlargement. More recently Bain and Robertson (1951) and Robertson and Turner (1951) have reported that fruit size differences within a tree were due mostly to variation in cell number and only to a small extent to variation in cell size.

Fruit size is also related to crop size. Apple trees usually bear a light crop, i.e. fewer fruits of larger size, and a heavy crop, i.e. more fruits of smaller size, in alternate years. This phenomenon is known as biennial bearing. Martin and Lewis (1952) found that differences in fruit size between light and heavy crop fruit is due mainly to differences in cell size rather than in cell number. Smock (1949) showed that in gas storage light crop fruit was more susceptible to brown core. A similar relation in the case of brown heart has been demonstrated by Martin and Carne (1950).

Martin (1954) has reported that light crop fruit is not only larger, but also has higher acidity, an earlier skin colour change from green to yellow, and later starch conversion than heavy crop fruit. Bitter pit was correlated with fruit size and with acid content, while the correlation between breakdown and fruit size was extremely high. The mean fruit diameter per tree was considered to be by far the

best index of the physiological behaviour of the fruit.

Seed number

While the pomologist and technologist are interested in the edible portion of the fruit rather than the seed, Smock and Neubert (1950) state that most varieties require seed production for fruit development. Exceptions to this rule are a few varieties such as Wilson's Seedless and Baldwin, which is seedless when not cross-pollinated. These authors believe that apples must have three to five seeds as a minimum number for fruits to be developed. A full complement of seeds is needed not only for fruit setting but also for well-shaped fruit. For example, when a McIntosh apple has four seeds out of a possible ten, and they are located in adjoining carpels, the fruit is larger on that side than on the seedless side. Kobel (1954) has pointed out that there is a consistent tendency for the larger fruits of a tree to have more seeds than smaller fruits. Tydeman (1952) showed a correlation between fruit weight and embryo weight. This relationship has been examined in greater detail by Rudloff and Schmidt (1953), and also by Schander (1955), who demonstrated that the relationship was an extremely complex one influenced by three groups of environmental factors, viz., a complex of general growth influences, the relative tendency to parthenocarpy, and the influence of competition between seeds. These workers did not investigate the relation of seeds to fruit physiology other than growth.

A few workers have reported a relationship between seed content and the incidence of storage disorders. Heinicke (1920) found that fruit affected with tree pit had lower seed content, and a greater proportion of poor seed, while the reverse was true of fruit of the same tree affected with storage pit. More recently Poapst and Phillips (1958) have reported a connection between seed physiology and the incidence of core flush. Seeds from fruits with core flush germinated more readily than seeds from sound fruits, while fruits inoculated with extract of stratified sound seeds had increased susceptibility to core flush.

Martin, Lewis and Cerny (1961) have found that in the absence of other disorders there is a positive intercorrelation between mean fruit size, susceptibility to Jonathan spot, and mean seed number per fruit both within and between trees. In a given fruit size group on a tree, fruits with Jonathan spot have a higher mean seed number than sound fruits, and these seeds have a greater tendency to germinate. Within a tree, thinning practices which result in fruits of different size but with the same seed number give no difference in Jonathan spot susceptibility. Between trees, thinning which produces fruits of the same size but different seed number results in a different level of Jonathan spot susceptibility. These workers have put forward the hypothesis that competition between seeds and fruit during the cell division stage may aggravate weaknesses of the respiratory mechanism which in

storage result in the formation of toxic by-products which cause the lesions.

Cell size

Smith (1940) determined the cell size and cell number of several varieties of English apples, and has related size and number of cells in mature apples to fruit size. There was an apparent correlation between the respiration rate and cell number when both were expressed on a fresh weight basis. Smith further pointed out that those varieties characterized by a greater number of cells and a higher respiration rate per unit weight were also those with characteristically poorer keeping quality.

Martin and Lewis (1952) have shown that, between varieties, cell volume, respiration per cell, protein nitrogen per cell, and respiration rate per unit of protein nitrogen are positively intercorrelated.

Colour

The colour of apple fruits is due to the presence of chlorophyll and carotenoids. In red varieties anthocyanins are also present. Yellowing of the skin during ripening is due to the destruction of the chlorophyll. The green or yellow colouration is called the ground colour, while the overlying red colouration, when present, is termed the surface colour. Although ground colour has been used as an index of maturity in apples, there has been little attempt to

correlate ground colour with pigment concentrations.

Haller and Magness (1926) have shown that ground colour is affected by the leaf : fruit ratio. Fewer leaves per fruit resulted in a green ground colour at harvest. This is the condition obtaining in fruit from a heavy crop tree.

Magness and Diehl (1924) observed that a skin coating consisting of a mixture of mineral oil and paraffin wax considerably retarded the change in ground colour. They considered this to be associated with increase in the resistance of the skin to gaseous diffusion. This was in accord with the later findings of Trout et al. (1942) and Hackney (1943a) that the rate of colour change of untreated apples was related to the internal gas concentration. More recently Trout et al. (1953) reported that coating apples with shellac and a number of different oils and waxes retarded yellowing. At higher temperatures the differences in rates of yellowing became more significant.

Colour change has been reported by Martin (1954) to be more rapid in dry seasons than in wet ones. Within one season a change from dry to wet conditions slowed the colour change, and vice versa. Colour change was much slower in fruit from deep well-watered soils especially in drier seasons, and this was accompanied by a lower content of soluble solids.

Respiration rate

The respiration rate of apple fruits during their development is not constant. Krotkov (1941) and Shaw (1942), working with McIntosh and Jonathan respectively, have shown that the rate of respiration is very rapid during the period just subsequent to fruit setting. It declines rapidly in early summer, and more slowly during late summer. Smock and Neubert (1950) have pointed out that the period during which respiration rate declines rapidly corresponds with the period of cell multiplication in the young fruit. Following the minimum on the respiration curve a rise occurs. The rate increases to a peak which is followed by a gradual decline. Kidd and West (1930, 1945) refer to this maximum rate as the "climacteric", and the subsequent phase of decline as "senescence".

Internal atmosphere composition

Several different methods have been used in the determination of oxygen and carbon dioxide concentrations in the internal atmosphere of fruits. Magness (1920) extracted the intercellular gas from cut apple tissue with a vacuum, and analyzed the samples with a Bonnier-Mengin apparatus. Claypool (1938) developed a method for the determination of total carbon dioxide, i.e. both in solution in the water of the tissue and in the atmosphere of the intercellular spaces. The tissue was boiled with acid, and the carbon dioxide

liberated was absorbed in standard alkali and estimated by titration. Using this method Claypool found that the amount of carbon dioxide present in fruits bore a close relationship to solubility curves for carbon dioxide in water. In studies on the internal atmosphere of tropical fruits, Wardlaw and Leonard (1936) made a hole with a cork borer into the centre of the fruit, inserted a sterilized glass tube and sealed around it with wax. After a suitable time a sample was transferred from the glass tube to a Haldane apparatus for analysis. Using this technique, Wardlaw and Leonard (1939, 1940) were able to detect, during the life of banana and papaw fruits, marked changes in internal oxygen and carbon dioxide levels which were associated with changes in both respiration rate and resistance of the tissue to gaseous movement. A method developed later by Smith (1947) for analyzing the internal atmosphere of apples possessed the advantage that a sample could be drawn from any part of the apple, at relatively short time intervals, and with very little injury to the tissue. The sample was taken by inserting a hypodermic needle into the apple through a thin sheet of rubber sealed with wax to the surface of the fruit. The rubber sheet prevented tearing of the tissue by the needle, and acted simultaneously as a gas-tight seal. The needle was connected with a gas pipette filled with mercury, and the gas sample was drawn out by the vacuum created by lowering the level of mercury in the pipette. No appreciable difference

in the composition of the internal atmosphere was observed which could be attributed to differences in the duration of the period of extraction, which was varied from one minute to 16 hours.

Using a modification of the gas-sampling technique of Wardlaw and Leonard (1936), Trout et al. (1942) found that increase in temperature caused a decrease in internal oxygen concentration and an increase in internal carbon dioxide concentration in Granny Smith apples. Earlier, Magness (1920) had shown this to be true for Yellow Newtown apples. At lower temperatures the sum of the percentages of oxygen and carbon dioxide was equal to 21%, the value for air, but at higher temperatures this total gradually increased. Magness suggested that this was probably due to the occurrence of anaerobic respiration, which would cause an increase in the concentration of carbon dioxide without a corresponding decrease in oxygen.

Gerber (1897) reported on the work of Frony (published in 1840 and 1860) who followed the internal atmosphere composition of apples through development and ripening. Oxygen concentration was higher in green fruit and decreased as the fruit matured on the tree. More recent work by Hackney (1944) indicated that there was a slight fall in internal oxygen level and a slight rise in internal carbon dioxide level during development. In apples which had been

stored at 0°C for two months Trout et al. (1942) found a marked falling off in oxygen level with age at 21°C, while the level of carbon dioxide remained fairly constant. During prolonged cool storage the internal oxygen concentration gradually declines (Hackney 1943, Trout et al. 1941) but there is no appreciable increase in carbon dioxide level.

The effects on internal atmosphere composition of applying coatings of various substances to the apple skin have been studied by a number of workers. Claypool (1938) observed that apples treated with a wax coating had a higher carbon dioxide content immediately following waxing, but this subsequently declined to a level which in some cases was lower than that in untreated fruit. Trout et al. (1941, 1942) found that coating the skin with various materials accelerated the fall in internal oxygen concentration at the onset of ripening. When internal oxygen level in skin-coated Granny Smith apples fell below 3%, a disorder identical to brown heart developed, although the internal carbon dioxide level was not significantly increased. Alcoholic fermentation and internal disorders did not always develop after treatment, and in some cases skin coating prolonged storage life. A depression of internal oxygen concentration in treated fruit has also been reported by Hackney (1943a) who used a castor oil - shellac coating.

Several workers have been engaged in endeavouring

to locate the resistance to gaseous exchange between the apple fruit and its environment. Trout et al. (1942) considered that the resistance was located mostly in the skin, since they did not find any gradient in internal atmosphere composition across the fruit. However, Smith (1947) found the internal carbon dioxide concentration to vary inversely with the distance from the centre of the fruit, while the oxygen concentration varied directly. A concentration gradient in both carbon dioxide and oxygen from the surface to the centre of an apple has also been observed by Ulrich (1956). He described a gradient in the permeability of the fruit tissue to gases, which decreases in the outer regions, particularly at the level of the cuticle. A gradient was also found in the distribution and form of lenticels on the surface. The lenticels in ripe Granny Smith apples are non-functional (Hall et al.1955) and since there exists normally no passage connecting the carpellary cavity to the calyx, exchange of gases must operate by diffusion across the skin. On the other hand Marcellin (1955, 1956) has shown that the lenticels are the main factor controlling the entry of oxygen into the tissue of the Calville apple, although they do not appear to play any particular role in the emission of carbon dioxide. The latter occurs by diffusion across the cuticle.

Marcellin (1954) has studied changes, in the course of fruit development, in the porosity of the tissue as

indicated by the volume of intercellular spaces. Porosity increased during growth, but decreased during maturation due to pectin obstruction of spaces. During senescence pprosity again increased due to resorption of the pectin of the cell walls.

Hackney (1944) found slight increases during fruit development in the resistance of the skin to diffusion of both oxygen and carbon dioxide. An increase in the resistance of the skin to gaseous diffusion accompanies ageing of the fruit (Trout et al.1942, Hall et al.1955), and has been attributed to an increase in the wax content of the skin. While prolonged cool storage has been shown by Trout et al. (1941) and by Hackney (1943) to bring about an increase in the resistance of the skin to the diffusion of oxygen, no marked change in the resistance to carbon dioxide diffusion has been observed. A castor oil-shellac coating was found by Hackney (1943a) to increase the resistance of the skin to carbon dioxide diffusion in apples removed from cool storage.

Trout et al.(1942) found that the respiration rate was positively correlated with the internal oxygen concentration, especially when the fruit was treated with a waxy coating, which brought about a reduction in both. In Granny Smith apples stored for less than four months, the respiration rate appeared to be governed by the internal oxygen concentration (Hackney 1943). However, this was not the case in fruits of this variety stored for more than five months, or in Delicious apples (Hackney 1944a).

Dry weight

Askew (1935) has shown that the dry weight curve during the development of the apple fruit is of the usual sigmoid type. The increase in dry weight during development is due largely to increase in carbohydrates (Fraser 1951).

Nitrogen

Attempts to find a relationship between the total nitrogen content of apples and their keeping quality have yielded conflicting results, probably because the factor of fruit size has been ignored in much of the work. While some workers (e.g. Archbold 1925, Collins 1957, Tiller et al. 1959) have found that soil treatments which resulted in high fruit nitrogen levels caused an increase in the susceptibility of the fruit to disorders in storage, others (e.g. Gourley and Hopkins 1931, Baxter 1958) have found no such effect.

Where attention has been directed rather to the alcohol-insoluble nitrogen fraction, commonly referred to as protein nitrogen, some interesting information has emerged. While there is no change in the total nitrogen content of an apple after harvest, marked changes have been shown to occur in the level of protein nitrogen (Archbold 1925, Hulme 1937). The rise in the percentage of protein nitrogen follows the respiratory rise, and the peak is reached shortly after the

climacteric peak in respiration rate is attained. Increase in protein nitrogen is accompanied by a decrease in soluble nitrogen. Kidd et al. (1939) found that ethylene stimulated not only respiration but also changes in the nitrogen fractions in the apple. They found too that increase in protein nitrogen content could be delayed by cool storage in a carbon dioxide enriched atmosphere.

Martin and Lewis (1952) found that in a number of varieties light crop fruit had a higher rate of respiration per unit of protein nitrogen than that of heavy crop fruit. They suggested that the more rapid senescence, and greater susceptibility to disorders, of light crop fruit might be related to this fact. Since cells of light crop fruit appeared to require more energy from respiration to maintain their protein, it was suggested that perhaps these cells are unable to maintain an efficient transfer of energy at the higher rates required. These workers offered an explanation for the observation that large fruit from heavy crops has a lower susceptibility to disorders than light crop fruit of the same size. The large size of the heavy crop fruit is due to increase in cell number rather than in cell size, and therefore the respiration rate per unit of protein nitrogen is lower. Robertson and Turner (1951) had considered that large fruit might have difficulty in maintaining cell constituents where high protein content was making severe demands on the

energy distributors of the cells. The work of Martin and Lewis (1952) showed that, although protein synthesis keeps pace with cell enlargement, the respiration rate per unit protein increases with cell size.

Soluble solids

The soluble solids content of apples is determined with a refractometer on the expressed juice, and calculated on the assumption that sucrose constitutes the major fraction of the total soluble solids.

Martin (1954) has demonstrated the variation in soluble solids level with variety and season. High rainfall appears to lower the level, while sunshine above normal appears to increase it. The level increases throughout the growing season. In studies over a number of years on Cox apples Martin found that in seasons of abundant sunshine soluble solids ranged from 11.0% in January to 16.5% in April, and in seasons of high rainfall from 9.5% to 13.0% over the same interval. Smock and Boynton (1944) have reported lower soluble solids levels in the fruit from orchards characterized by a high level of nitrogen in the soil. Haller and Magness (1926) suggest that the fruit : leaf ratio may affect the amount of sugars in the fruit. Smock (1941) has demonstrated that fruits from limbs from which all the leaves have been removed may reach full size but have a lower sugar content. Smock (unpublished) does

not exclude the possibility that sugars may be manufactured to a small extent by the fruit itself through photosynthesis, since fruits shaded with paper did not accumulate as much sugar as did unshaded fruits.

Archbold (1932) has reported an increase in sugars after harvest for at least a short period, due to the hydrolysis of starch and to conversion from other non-sugar materials. Following this there was a gradual decline due to respiration. Miller and Brooks (1932) and Miller and Dowd (1936) found that storage of apples in an atmosphere high in carbon dioxide slowed down the loss of sugars.

Free acids

While malic acid is the predominant acid in apple fruits, various other acids have been reported to be present in much smaller amounts. A few weeks after fruit set the acid concentration begins a gradual decline which persists up to the time of harvest (Archbold 1932, Bigelow 1906, Caldwell 1934). Magness et al. (1926) and Haynes and Archbold (1928) have observed this decline to continue after harvest. Their explanation was that acids served as a partial substrate for respiration along with sugars. Lowering the temperature of apples resulted in a retardation of the acid loss. Smock (unpublished) has observed that this loss may also be retarded by a high carbon dioxide level or a low oxygen level in the cool storage atmosphere.

Acidity varies with variety and season. In a range of varieties Fellers (1928) found concentrations ranging from 0.38% to 1.11%. Caldwell (1928) observed that in a season with an abundance of intense sunshine the acidity, as well as the sugar content, was high. Haller and Magness (1926) have reported an effect of leaf : fruit ratio on the acid content of apples. This indicates that acidity depends partly on, or is correlated with, carbohydrate manufacture by the leaves.

Plagge and Gerhardt (1930) showed that the incidence of Jonathan spot was higher in fruit which had a low acid content at the beginning of the storage period, and in those apples which showed the smallest acid loss during storage.

Bitter pit

This disorder has been described in detail by Brooks and Fisher (1918). It has been detected in virtually all varieties at one time or another but in Tasmania is most prevalent in Cox, Cleopatra, Sturmer, Granny Smith and Gravenstein. It has been a subject of much study over a long period. In 1934 Barker reviewed 209 papers concerning bitter pit published during the preceding 65 years in an attempt to put some order into the mass of results and observations that had accumulated. He concluded that there was no critical information regarding the causal

factors, and that all that was known were certain characteristics of the disorder. The following facts were found to be almost universally accepted:-

(a) liability to develop pit after picking decreases with increasing maturity at picking (without the development of an equivalent amount of tree pit);

(b) susceptibility increases with increasing fruit size;

(c) fruit from light crops, from young and vigorous trees, and from those given heavy nitrogen dressings have high susceptibility.

More recent investigations have confirmed these findings. For example, Martin (1954) found bitter pit incidence to be correlated positively with fruit size and negatively with crop size. Smock (1941) applied heavy nitrogen dressings and injections of urea into the limbs after the June drop and increased the incidence of bitter pit.

The possible association of bitter pit with boron utilization has been studied in various parts of the world. Dunlap and Thompson (1959) have reported that bitter pit is associated with the boron metabolism of the plant, and that it can be effectively reduced by boron sprays, especially when applied during the blossoming period. However, Atkinson (1937), Cockayne (1937), Magness (1940, 1942), Maier (1941), Levy and Roach (1937) and Wallace and Jones (1940) have failed to control the disorder with boron

applied to the soil or injected into the tree.

The possible role of water stress as a factor in bitter pit incidence has received attention. Heinicke (1920) noted that on Baldwin trees the central fruits were less affected than the lateral fruits borne on the same spur. He surmised this to be due to the upper portion of the spur being better situated for obtaining water and nutrients. Smock (1937) was able to reduce pit by defoliation in early June, and explained this on the basis of competition between leaves and fruit for water. He found evidence for some maladjustment in the normal water relations of the fruit. Any treatment which accentuated the "pulling power" of the leaves for water (because of their advantage in osmotic concentration) at the expense of the fruits increased the susceptibility of fruits to bitter pit.

Two leads which were set comparatively early but were not followed up for many years have laid the basis for recent work which shows promise in relating bitter pit incidence to mineral balance. DeLong (1936, 1937) showed that pitted fruit had a lower calcium content than sound fruit, and concluded that there could be competition between leaves and fruits for calcium. Earlier, Rose et al. (1933) had observed that magnesium sulphate in irrigation water increased pit. It was not until 1956

that Garman and Mathis reported a reduction in pit incidence from sprays and soil injections of calcium salts, and an increase from magnesium applications. Their analytical work indicated that incidence was related to the balance between calcium and magnesium, or between calcium and magnesium plus potassium. The findings of Garman and Mathis have since been confirmed by a number of workers, and much study is now being directed towards the problem of mineral balance within the tree. Foliar application of solutions of calcium salts has become standard practice in combating this disorder.

Hall et al. (1953) found that pit was reduced by skin coatings, an alcoholic solution of castor oil and shellac giving the best results.

Jonathan spot

This is a disorder of red varieties, notably Jonathan and Spitzenberg, and to a lesser extent Rome Beauty, Scarlet and Worcester (Carne 1948).

Carne (1928) describes the spots as at first ill-defined, appearing dark red or brown on red skin and greenish yellow to brown on yellowish skin. They are located mainly on the cheeks of the fruit, and do not necessarily originate at lenticels. In time the spots become more defined, darker, and very slightly sunken.

At this stage they frequently show a dark ring border. They are not subject to fungal infection. At times the spots coalesce to form an irregular outline. They occur chiefly on the more highly coloured portion of the skin, and the best-coloured fruit in exposed situations is the most liable. Usually the spots are entirely superficial and do not extend into the flesh.

Pentzer (1925) has shown that the tissue immediately below the spots has a lower acidity than adjacent tissue. He suggested that this reduced acidity was responsible for the change in the anthocyanin pigments from red to blue, producing the characteristic spots. Plagge and Gerhardt (1930) produced evidence that Jonathan apples with higher acidity were less liable to Jonathan spot than were those in which acidity was allowed to diminish by delaying cool storage. All workers agree that liability to the disorder normally increases with increased maturity. Carne (1928) states that Jonathan apples held too long on the tree may develop Jonathan spot before picking. Generally speaking, however, it appears six to eight weeks after picking, and three to four months afterwards if the fruit has been in cool storage.

Martin et al.(1961) have demonstrated an interaction between Jonathan spot and breakdown in Jonathan apples. While there was a negative correlation between

them, the same fruits tended to be susceptible to both disorders. In the absence of other disorders there was a positive correlation between Jonathan spot incidence, mean fruit size, and seed number, both within and between trees.

The disorder may be suppressed by the use of controlled atmosphere storage. Martin and Cerny (1956) found 5% carbon dioxide to be effective, while 0.2% carbon dioxide and 5% oxygen was far less effective but still better than air. Dewey et al.(1957) have reported good results with an atmosphere containing 2.5 - 5.0% carbon dioxide and 3% oxygen. Skin coating has also been used effectively for the control of this disorder (Hall et al. 1953).

EXPERIMENTAL MATERIAL AND METHODS

Choice of material

Cox apples were harvested from an orchard at Cradoc in the Huon Valley. Two trees, one with a light crop and the other with a heavy crop, were chosen as near to each other as possible to avoid any influence of variation in soil. One and a half bushels of fruit were harvested at random from each tree on February 22, 1960. Two days later an additional half bushel was picked as randomly as possible from the heavy crop tree, since the first picking did not include a sufficient number of 2½" fruit. The fruit was graded for size immediately after picking. From the different size groups apples were taken for experiments designed to study the behaviour of single fruits. They were selected for normal shape and freedom from mechanical injury, russet and sun scald. The fruits were chosen in such a way that for each apple from the light crop tree there was a corresponding one from the heavy crop tree with approximately equal weight.

Two days after picking, gas pipettes were inserted into 32 selected fruits which were then placed in a constant temperature room operating at 20°C for internal atmosphere and respiration determinations. The remainder of the fruit was wrapped in plain paper wraps, placed in open factory boxes, covered with sheets of paper, and

stored at 1.0°C for subsequent examination for storage disorders.

Jonathan apples were harvested from an orchard in the Ranelagh district of the Huon Valley. Selection of trees, and picking and grading of the fruit, were carried out on the same principles as with the Cox variety. Two bushels were harvested from each tree on March 16, 1960, and graded immediately. Gas pipettes were inserted into 32 selected fruits as before, and the remainder were placed in storage.

In 1961 apples were harvested from the same trees as in the previous year. However, due to the alternation in the cropping of the trees, those trees which had borne a heavy crop in 1960 were carrying a light crop in 1961, while those which had borne a light crop now carried a heavy one. Cox fruit was harvested on February 23, 1961, and Jonathan fruit on March 14. Following harvest the fruit was subjected to the same selection and treatment as was that of the previous season.

Classification of fruits

In each year there were eight fruits in each of the following classes.

	Range of fruit weights (g)	
	1960	1961
Cox		
Light crop 2¼"	80.0 - 83.5	86.5 - 96.0
Heavy crop 2¼"	80.0 - 83.0	87.0 - 96.0
Light crop 2½"	97.5 - 105.5	108.0 - 117.0
Heavy crop 2½"	97.0 - 105.0	108.0 - 117.0
Jonathan		
Light crop 2¼"	81.2 - 84.7	75.0 - 79.8
Heavy crop 2¼"	81.2 - 84.8	75.0 - 79.8
Light crop 2½"	92.7 - 100.5	96.0 - 108.0
Heavy crop 2½"	92.5 - 100.0	96.0 - 108.0

Gas pipette for internal atmosphere measurements

The gas pipette is illustrated in Figure 1. Each pipette was fitted with a rubber seal at one end through which a small sample of gas was drawn off by means of a hypodermic needle fitted onto a Haldane gas analysis apparatus. Fitted near the other end of the pipette was a rubber washer to provide an airtight seal between the pipette and the fruit. Before insertion the pipette, complete with rubber seal and washer, was sterilized in an oven at 150°C.

To connect the pipette with the central cavity of

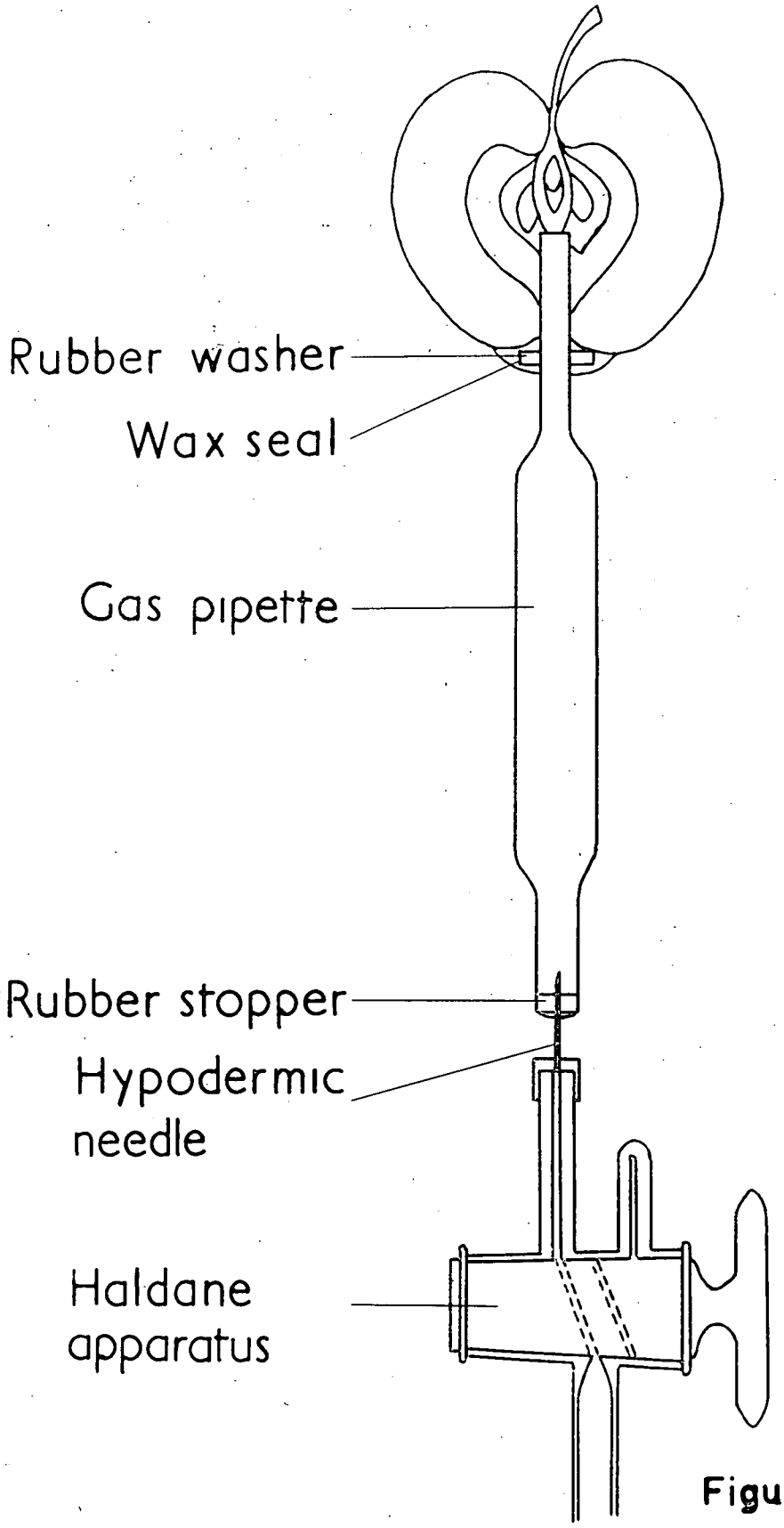
Rubber washer
Wax seal
Gas pipette
Rubber stopper
Hypodermic needle
Haldane apparatus

Figure 1

the fruit it was inserted through the calyx, because the depression at the calyx helped to make the seal strong and rigid. Insertion through the calyx also eliminated the potential source of error in fruits with an open calyx which might provide an air leak to the central cavity.

After sterilizing the calyx with cotton wool soaked in alcohol, a hole was made with a cork borer previously sterilized in a flame and used while still hot. The end of the pipette was then sterilized in the flame and inserted in the hole made by the cork borer. Melted beeswax was used to seal around the junction between the pipette and the fruit. With careful sterile handling the possibility of fungal infection was small. Only one of the 64 apples treated in this way in 1960 developed a rot.

The attachment of the gas pipette directly to the apple had the following advantages:

(1) All the handling of the fruit was done with the pipette, so that the apple did not need to be touched by hand at any time during the experiment.

(2) Additional infection was practically impossible since there was no direct connection between the internal and external atmospheres.

(3) For the measurement of oxygen uptake the fruit was suspended on the pipette which was inserted in the rubber stopper which closed the respiratory vessel (See Figure 2).

For storing the apples fitted with gas pipettes, wooden stands were used. These consisted of 2" thick wooden boards supported by 2" high legs, and drilled with holes of such a size that the gas pipettes could be inserted and stand erect.

The fruit was stored in a constant temperature room at 21°C for 65 hours. This time was found to be adequate for the return of the apple to normal metabolism following injury by the cork borer, and also for the atmosphere in the pipette to come into equilibrium with that in the central cavity of the fruit.

Measurement of the internal atmosphere

Determinations were made of the concentrations of both carbon dioxide and oxygen in the central cavity of the apple fruits. Measurements were made with the Haldane apparatus on the principle followed by Wardlaw and Leonard (1939), by Trout et al.(1942) and by Hackney (1943). The only difference between the method described by Trout et al. (1942) and that used in the present studies was that the sample of gases from the apple was drawn out from the gas pipette by means of a hypodermic needle fitted to the Haldane apparatus. That end of the gas pipette which was sealed with a rubber disc was pushed onto the hypodermic needle which was previously filled with mercury from the Haldane burette. By lowering the mercury column the sample was

drawn into the Haldane apparatus. The capacity of the apparatus used was 10cc, the burette having coarse graduations from zero to 7cc, and 0.01cc graduations for the remaining 3cc. For this reason it was necessary to mix the sample of gas from the apple with 7cc of air, previously freed from carbon dioxide and oxygen, to make possible an exact reading on the finely graduated portion of the scale.

The actual determination of carbon dioxide and oxygen was carried out in the following manner.

Part of the carbon dioxide-and oxygen-free air remaining from the previous analysis in the burette of the Haldane apparatus was forced into the atmosphere through the hypodermic needle, so that exactly 7 cc was left. After adjusting the hairline indicators on the capillary tubes above the vessels containing the potassium hydroxide and alkaline pyrogallol, the tap on the compensating vessel was turned to allow it to come to equilibrium with the barometric pressure. This tap was then closed and the three-way tap from the potassium hydroxide vessel was turned so as to connect this vessel with the burette. The mercury column in the burette was then raised to force the 7cc of carbon dioxide- and oxygen-free air into the potassium hydroxide vessel. The three-way tap on the top of the burette was then turned so as to connect the burette with the hypodermic needle. The mercury level in the

burette was raised sufficiently to fill completely the arm with the hypodermic needle. The gas pipette attached to an apple was pushed onto the hypodermic needle so that the tip of the needle passed through the rubber stopper into the pipette. By lowering the mercury level, approximately 2.5cc of gas was drawn into the burette. The three-way tap was then turned to disconnect the burette from the arm with the hypodermic needle and to connect it with the potassium hydroxide container. The mercury column was lowered until the level of the potassium hydroxide coincided with the hairline indicator. The reading on the burette was then recorded, this indicating the volume of the gas sample from the apple plus the 7cc from the potassium hydroxide vessel. This mixture was washed four times through the potassium hydroxide. Following this the reading of the burette gave the value from which the percentage of carbon dioxide was calculated. The three-way tap from the potassium hydroxide container was turned to connect the burette with the alkaline pyrogallol vessel. The gas mixture was then washed through the pyrogallol until the reading was constant. From this reading the percentage of oxygen was calculated.

The following formulae were used to calculate the percentages of carbon dioxide and oxygen.

$$\% \text{ carbon dioxide} = \frac{B - C}{B - A} \times 100$$

$$\% \text{ oxygen} = \frac{C - D}{B - A} \times 100$$

where

A = initial volume of air free of oxygen and carbon dioxide.

B = A plus volume of gas sample from apple

C = A plus B with carbon dioxide absorbed

D = C with oxygen absorbed.

Measurement of the respiration rate

Respiration rate was measured with the respirometer developed by Sykes (1944) and modified to meet requirements. With this method the carbon dioxide produced by the fruit is absorbed by normal sodium hydroxide solution. Constant pressure is maintained by replacing the oxygen taken up by the fruit by a column of mercury. The apparatus, which is essentially a differential volumeter, is illustrated in Figure 2. Two airtight containers, a compensating chamber, A, and a respiratory chamber, B, are connected by means of a capillary tube of 1mm bore, C. This tube contains a drop of kerosene coloured with Waxoline Yellow I. S., which serves as a zero pressure indicator. An adjustable hairline on the capillary can be moved so that at the start of the measurement it coincides with one end of the kerosene drop. The best length for the drop is $\frac{1}{8}$ - $\frac{3}{8}$". The kerosene may be

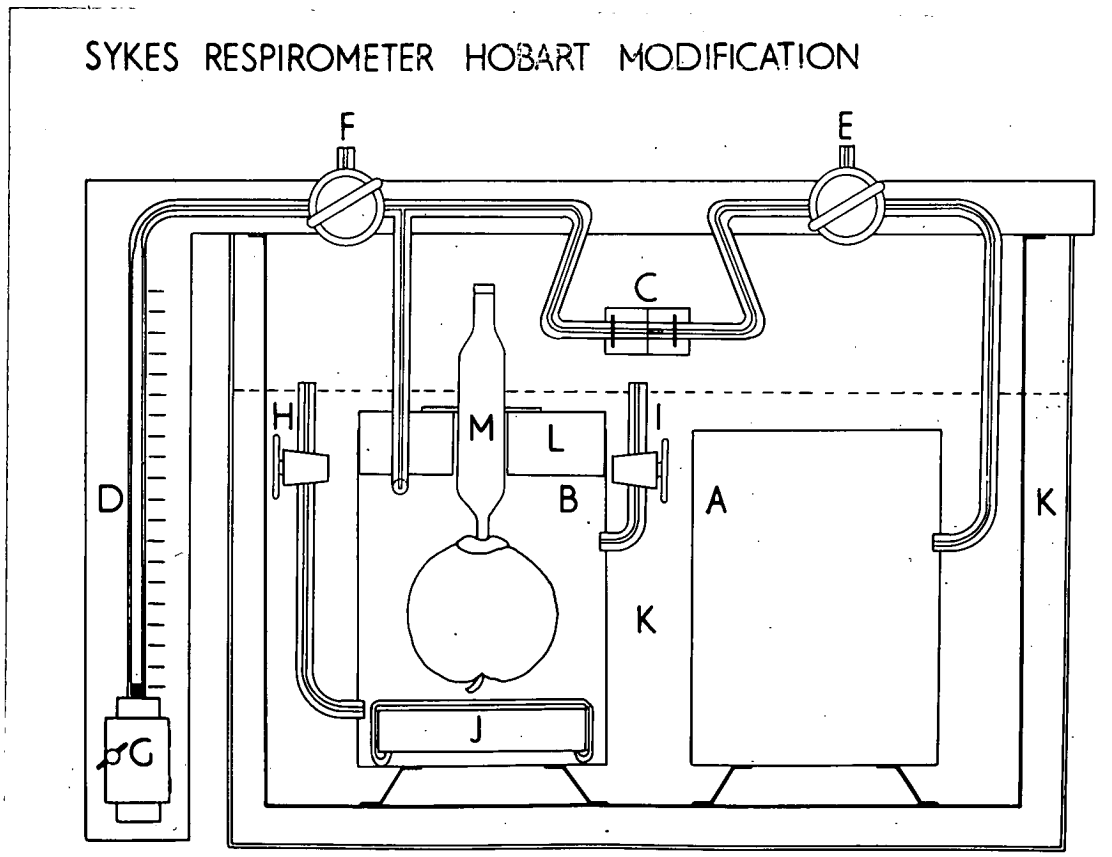

Figure 2

injected into the zero pressure indicator through the three-way tap, E, using a syringe and very thin plastic tubing. The respiratory chamber has two taps, H and I, opening to the atmosphere. For chambers A and B, 30 oz cans, 4 1/16" in diameter and 4 11/16" high, are used. Chamber B is first sealed and then opened on the top end with a can opener. The resulting seam imparts extra rigidity to the container. The chamber is sealed with a large rubber stopper The connections between the glass capillary tubing and the copper tubing soldered into the cans are made with rigid plastic tubing, as are all connections between capillary tubing, taps and burette. The chambers are fastened to a U-shaped platform 11½" long, 9" high, and 7" wide, constructed of 16 gauge galvanized iron sheet.

The burette is made from a 2cc graduated pipette and two three-way taps, E and F. It is attached to an L-shaped wooden frame made from 2" x 1" hardwood screwed to the metal platform. The three-way tap, F, is used as a connection between the burette, the respiratory chamber B, the zero pressure indicator, C, and the atmosphere. The three-way tap, E, forms a connection between the compensating chamber A, the zero pressure indicator and the atmosphere. The burette, D, contains a column of mercury, the level of which is adjusted by means of a screw clip, G, compressing the rubber tubing at the end of the burette.

The CO_2 absorbent, 10ml N NaOH, is placed at the bottom of the respiratory chamber in a Petri dish to which is attached a bridle of thick plastic-covered wire. The Petri dish may be lowered or taken out by gripping this bridle with forceps.

Because this method is based on volume changes at constant pressure, the temperature of the system is kept as constant as possible by immersing the respirometers in a vigorously stirred water bath in a constant temperature room.

The following procedure is followed for the measurement of respiration.

(1) The taps F, E, H, and I are open.

(2) The kerosene drop in the zero pressure indicator is moved to the middle by raising or lowering one end of the respirometer. Tap E is turned to connect the compensating chamber with the atmosphere, at the same time sealing off the zero pressure indicator. This prevents drifting of the kerosene drop.

(3) The Petri dish containing the sodium hydroxide solution is placed at the bottom of the respiratory chamber.

(4) An apple is placed on a wire platform in the respiratory chamber.

(5) The respiratory chamber is sealed with a rubber stopper, L, which is moistened with a damp cloth. The moistening of the stopper has proved to be essential for a perfect seal.

(6) The respirometer, with taps F, H and I still open to assure good circulation of air between atmosphere and respiratory chamber, is lowered into the water bath and left for a minimum of 2½ hours to come into equilibrium with the water temperature. (Perfect equilibrium is never reached, the air temperature inside the respiratory chamber remaining constantly 0.3°C higher than the water temperature).

(7) The mercury column in the burette is adjusted with the screw clip to the zero position.

(8) Fortyfive seconds before zero time, taps H and I are shut.

(9) Thirty seconds before zero time tap E is turned for five seconds so that the zero pressure indicator, the compensating chamber and the atmosphere are all connected. It is then turned so that the zero pressure indicator and the compensating chamber are connected and the connection to the atmosphere is shut off. This usually causes slow drifting of the kerosene drop in one direction or the other.

(10) Fifteen seconds before zero time tap F is turned so that the burette is connected with the respiratory chamber and the zero pressure indicator, and all components are shut off from the atmosphere.

(11) The hairline on the zero pressure indicator is moved to such a position that at zero time it is exactly on the meniscus of the kerosene drop. The pressure reduction created during the 15 seconds between shutting tap F and adjusting the hairline is so small that its effect on the rate of respiration of the fruit is neglibible. At zero time the respiration rate actually begins to be indicated by the drifting of the kerosene drop in the direction of the respiratory chamber.

(12) By screwing the clip G the mercury is pushed into the burette D and replaces the oxygen taken up by the apple, while the carbon dioxide evolved is absorbed by the sodium hydroxide.

(13) Every ten minutes for a period of one hour the mercury column is adjusted so that the meniscus of the kerosene drop exactly coincides with the hairline, and the reading on the burette is recorded. This gives the total oxygen uptake per fruit per hour, while the six separate readings indicate the degree of fluctuation.

(14) After the last reading tap E is turned to shut off the zero pressure indicator and to connect the compensating chamber with the atmosphere. Drifting of the kerosene drop is thus prevented.

(15) Tap F is turned to connect the burette, the respiratory chamber and the zero pressure indicator with the atmosphere.

(16) Taps H and I are opened and the mercury column is lowered back to zero.

(17) After 30 minutes the procedure may be repeated. This time lapse between finishing the first respiration measurement and commencing the second is sufficient to allow the atmosphere in the respiratory chamber to come into equilibrium with that outside.

The procedure may be modified for the measurement of the respiratory quotient (R.Q.). Instead of being absorbed in sodium hydroxide solution the carbon dioxide produced is allowed to accumulate in the respiratory chamber. The column of mercury in the burette is set before commencement on the 1.00ml mark, i.e. at the middle of the burette, so that an increase or decrease in the volume of gases in the respiratory chamber may be measured. If any decrease occurs in the volume of the atmosphere in this chamber the kerosene drop will drift in the direction

of the respiratory chamber, indicating an R.Q. less than unity. If an increase occurs the drop will drift towards the compensating chamber, indicating an R.Q. greater than unity. Water temperature and atmospheric pressure are recorded after measurements have commenced.

Calculations were made using the following formulae:

(1) Oxygen uptake

Rate =

$$\frac{X \times 60}{m} \times \frac{273}{273 + t} \times \frac{P}{760} \times \frac{32}{32.4} \times \frac{10^4}{W} \text{ mg/10kg / hr}$$

where X = decrease in volume of atmosphere in respiratory chamber read on burette (cc)

m = length of observation period (min)

p = barometric pressure (mm)

t = bath temperature (^{o}C)

(2) Respiratory quotient

$$R.Q. = \frac{\frac{X \times 60}{m} + \frac{X_1 \times 60}{m}}{\frac{X \times 60}{m}}$$

Cell size determination

Tissue was taken from each fruit by inserting a cork borer of approximately 8 mm diameter through the midcortex parallel to the axis of the fruit, in the middle of the sun-reddened side. A second piece of tissue was taken from a position opposite the first, a third piece 90^{o} from the second, and a fourth opposite the third, so that four evenly spaced samples were taken from each fruit. From the mid-section of each cylinder of tissue a disc was cut with two razor blades, fixed firmly side by side 2mm apart.

The discs were evacuated and kept until needed in a fixative solution of the following formula:

50% ethanol	100ml
formalin	6.5ml
acetic acid	2.5ml.

When cell size was to be determined, the fixative was drained from the discs and they were thoroughly rinsed with water before being placed in a macerating solution made up as follows:

ethylenediaminetetraacetic acid	20g
urea	7.5g
n-butanol	50ml
2N sodium hydroxide	50ml
water	to make one litre.

Twenty millilitres of this solution were added to each sample prior to incubation for 16 hr at 45°C.

Following incubation the discs were placed in a Waring blendor, the blades of which were reversed to prevent rupturing of the cells. Sufficient water to cover the blades was added and the mixer was run at half speed for one minute. The homogenate was transferred to a beaker and allowed to stand until the cells had settled. After pouring the surplus water from the beaker the cell suspension was placed in a specimen tube. As a preservative a crystal of thymol was added, or alternatively sufficient ethanol

was added to bring its concentration to 50%. The cells were stained with Ruthenium red or Bismark brown, 5ml of 0.1% dye being added to the cell suspension which was approximately 25ml in volume. After shaking up the cells, a sufficient quantity of the suspension was pipetted onto the chamber of a haemocytometer to fill the 0.2mm-deep cell. The haemocytometer was placed under a microscope and, using green light, two fields from each sample, each containing 150 to 200 cells, were photographed on 35mm Adox KB14 film using an aperture of f/2.8 and an exposure time of two seconds. The magnification of the cells was determined by photographing a stage micrometer.

After development the negative film was projected onto a screen. Care was taken to ensure that the light from the projector was centred and did not distort the size of the cells. The longest axis and the widest axis perpendicular to the first were measured for each of 50 cells in each field. A grid on the screen helped to prevent duplication of the measurement of any cell. The cells were treated as spheroids for calculation of their volume from the formula

$$\text{Volume} = \frac{4}{3} ab^2$$

where a and b are half the lengths of the major and minor axes respectively.

In order to determine the mean number of cells per fruit, the following formula was used:

$$N = \frac{W}{\text{Sp. g. of cells} \times V}$$

where W is the weight of the fruit and V is the mean cell volume of the fruit. For the specific gravity of the cells, the value of 1.1 was used, based on the work of Smith (1938) with a number of apple varieties.

Seed number determination

During the initial preparation of the fruit for analysis the seed number was determined. The apple was cut transversely at the centre and the number of seeds in each of the following categories was recorded.

(1) Fully developed seeds of full size and thickness.

(2) Partly developed seeds, full size or almost full size, but flat.

(3) Undeveloped seeds, very small and flat.

In Cox apples only the seeds of the first two categories were counted.

Determination of soluble solids

This determination was carried out with the fresh tissue while sections were being cut for cell measurements. From each cylinder of apple tissue a small piece of standard size was taken. The refractive

index of the juice extracted by squeezing the four pieces from each fruit was determined with an Abbe refractometer. From this value the soluble solids content of the fruit was calculated on the assumption that sucrose constituted the major fraction of the total soluble solids.

Determination of free acids

Fresh apple tissue was used for this estimation also. The remaining tissue from the four cylinders was freed of peel, placed in a glass weighing bottle and weighed. The tissue was then disintegrated with 30ml distilled water in a small stainless steel homogeniser jar for one minute. Any pieces of tissue adhering to the bottom of the jar were then loosened with a piece of fine wire, and the homogeniser was run for a further minute. Twentyfive millilitres of the resulting liquid were pipetted into a small beaker and enough distilled water was added to allow proper stirring. Free acids were titrated with 0.1N sodium hydroxide from a 5ml burette, using phenolphthalein as indicator. The following formulae were used for the conversion of the titre to free acids percentage (expressed as malic acid).

$$\text{Juice equivalent (ml)} = \frac{\text{weight of tissue (g) x 25}}{30 + (\text{weight} \times \frac{\text{Moisture content}}{100})}$$

$$\% \text{ free acids} = \frac{\text{Titre (ml) x normality of NaOH x 0.067}}{\text{Juice equivalent (ml)}}$$

Determination of moisture content

After the sections had been cut for the determination of cell size, free acids, and soluble solids, the apple was carefully peeled, the core was removed, and the rest of the tissue was cut into thin slices. These were placed in a moisture can lined with fine wire mesh to prevent the slices from sticking to the bottom of the can. Immediately after weighing, the samples were placed in a tunnel drier with a strong current of air at 65°C. The material was dried to constant weight within 16 hours and then weighed. The loss in weight of the tissue during the drying process was used for the calculation of the percentage of moisture and dry matter in the flesh of the fruit. Later the material was ground with a small mortar grinder to a fine powder, which was stored for subsequent analysis in a glass jar sealed with paraffin wax.

Nitrogen determination

Total nitrogen was determined by the Kjeldahl method. About one gram of the dried powder was digested in a 250ml round bottom flask with 8ml concentrated sulphuric acid. A small amount of potassium sulphate

was added to raise the boiling point of the acid, while selenium powder and copper sulphate were used as catalysts. The resulting digest was steam distilled in a Parnas-Wagner apparatus in the presence of excess sodium hydroxide. The ammonia liberated was absorbed in 10ml 1% boric acid solution and titrated directly with 0.01N hydrochloric acid using a mixed indicator composed of methyl red and methylene blue. The following formula was used for the conversion of the titre to percent nitrogen.

$$\% \text{ nitrogen} = \frac{1.4 \times \text{normality of HCl} \times \text{titre (ml)}}{\text{weight of sample (g)}}$$

For the determination of protein nitrogen a larger sample of about two grams of the dried powder was weighed in a small weighed packet made by folding an 18 cm Whatman No. 50 filter paper, and the packet was securely closed by means of sliding paper clips. The powder was then subjected to extraction with 75% ethanol in a Soxhlet apparatus for 16 hours, the extractant being replaced by fresh 75% ethanol after the first eight hours. This treatment has been shown by Hulme (1937) to remove all the soluble nitrogen constituents of the apple powder, and the residual nitrogen may be regarded as "protein nitrogen". After extraction the sample was dried at 40°C and transferred from the packet to a 250ml round bottom flask. From this point the procedure was identical with that described for the estimation of total nitrogen.

A value for the soluble nitrogen content was obtained by subtracting the value for the protein nitrogen content from that for total nitrogen content.

Evaluation of colour

After determinations had been made of internal atmosphere composition and oxygen uptake rate in the fruits held at 21°C, the apples were graded according to ground colour. This was possible only with the Cox fruits, as the colour of the Jonathan fruits was highly uniform.

The eight light crop fruits and eight heavy crop fruits of one size group were ranked according to ground colour from green to yellow, and numbered from 1 (most green) to 16 (most yellow). This was not difficult, and there was little doubt as to the classification of any apple. However it is possible that the differences between the three numbers at each extreme were very slight, with more significance in the differences in the middle of the series. Nevertheless it was the best possible grading for visual evaluation.

Evaluation of disorders

The only disorders which occurred were bitter pit in Cox fruit and Jonathan spot in Jonathan fruit.

The Cox apples were graded from 1 to 11, sound fruits

being designated as 1 and the most severely affected ones as 11. It was not possible to grade them in a continuous sequence because of a predominance of sound fruits, very lightly affected fruits, and very severely affected fruits. Therefore among the 16 apples there were some numbers missing, while others appeared several times.

The 16 Jonathan apples of each size group were graded for Jonathan spot from 1 to 16 because of the continuous variation in the intensity of the disorder. Again some of the lowest and some of the highest numbers showed only very slight differences in the intensity of the disorder.

Statistical treatment of results

All the data were subjected to statistical analysis by procedures recommended by Fisher (1932).

RESULTS AND DISCUSSION

To illustrate the big differences between the individual apples of one size, tree and year, the highest and the lowest results from only sound apples from the year 1961 have been arranged in the Table I.

Variety	Size & Crop		Seed No.	Dry Matter	Int. CO_2	Resp. Rate	Sol. Sols.	Free Acids	Tot. N.	Cell. Volume
Cox	2½" Light Crop	Highest	10	18.6	4.6	192	17.3	46	33	409
		Lowest	6	15.7	3.8	146	14.4	29	29	316
		Diff %	67%	18%	20%	32%	20%	59%	14%	29%
Cox	2½" Heavy Crop +	Highest	8	18.0	4.7	182	15.9	49	28	383
		Lowest	3	15.1	3.1	114	13.7	38	16	289
		Diff %	166%	19%	52%	60%	16%	29%	75%	33%

Tables 2 and 3 present the data obtained with Cox fruit in the years 1960 and 1961 respectively. The corresponding data for Jonathan fruit in the two seasons are presented in Tables 4 and 5. All differences between light crop and heavy crop fruit have been subjected to statistical treatment. In addition, all pairs of characteristics have been examined for correlations. Only those cases in which significance has been found will be discussed here. Some of the explanations put forward are speculative and open to criticism. They are included in view of stimulating further investigations, perhaps along different lines and using other methods.

June 1962.

THE UNIVERSITY OF TASMANIA

HIGHER DEGREES IN THE FACULTY OF SCIENCE

NOTES FOR THE GUIDANCE OF EXAMINERS

CANDIDATE: J. Cerny

DEGREE: Master of Science

SUBJECT OF THESIS: Some Relationships between Physiology and Storage Behaviour in individual Apples

EXAMINERS: Professor R.N. Robertson, University of Adelaide
Professor H.N. Barber, University of Tasmania

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Seed number

In the Cox variety, in both 1960 and 1961, the number of fully developed seeds per fruit was larger in light crop fruit than in heavy crop fruit. In the second season the difference was significant only in the fruit of the 2½" size group. These findings are in accord with those of Martin, Lewis and Cerny (1961), who reported a positive correlation between mean fruit size per tree and mean seed number. In the present investigations with Jonathan fruit, unexpectedly, there was no significant difference in seed number between light and heavy crop fruit in either year.

In 1960 the number of fully developed seeds per fruit was considerably higher in Jonathan than in Cox, while in 1961 the seed number was practically the same in each variety.

Smock and Neubert (1950) state that except in seedless varieties an apple requires, for normal development, a minimum of three to five seeds fairly evenly spaced in the carpel. In the present work it has been found that these seeds do not need to be fully developed to ensure perfect development of the fruit. Of the 32 Cox apples examined in this experiment in 1960, only 11 had three or more fully developed seeds, yet all fruits were perfectly shaped. The total number of seeds per fruit varied from

four to nine.

Cell volume and cell number

Light crop fruits were found to have larger cells and fewer cells per fruit than heavy crop fruits. The differences were significant in Jonathan in both years, but in Cox in 1960 only.

The smaller cell number of light crop fruits of a given size group when compared with heavy crop fruits may be due to a shorter period of cell division or to a lower rate of cell division or to a combination of both. Any of these three situations may be explained on either of the following grounds. There may be a shortage of cell division factors in the weak buds of a light crop due to the drain on the tree of the heavy fruit crop in the previous season. Alternatively there may be greater competition in light crop trees between developing leaves and fruitlets for compounds essential for cell division. This may be due not only to the larger number of leaves per fruit in light crop trees, but also to the fact that in such trees the time of onset of leaf development is earlier than in heavy crop trees, and coincides more closely with the period of cell division in the fruit.

The greater cell size of light crop fruits of a given size group is considered by Martin and Lewis (1952)

to be responsible for the greater susceptibility of such fruits to physiological disorders in storage, because of the higher rate of respiration required for the maintenance of a given amount of cellular protein. Differences in disorder susceptibility between light and heavy crop fruits in the same size group have been found to be directly related to differences in cell size even in the small samples used in the present investigations.

Colour

Colour was evaluated in Cox in both seasons, but in Jonathan only in 1961. In every case light crop fruits were yellower than heavy crop fruits, although the difference was not significant in Cox in 1961. These findings are in accord with those of Haller and Magness (1926), who demonstrated an influence of the leaf : fruit ratio on ground colour.

Respiration rate

In 1960 there was no significant difference in respiration rate between light and heavy crop Cox fruit. However, in 1961 the light crop fruit had a respiration rate almost 30% higher than the heavy crop fruit.

In 1960 Jonathan light crop fruit had a respiration rate more than 20% higher than that of heavy crop fruit, but in the following year there was no significant difference.

Martin (1954) measured the respiration rate of

Cox apples from a light and a heavy crop tree in two consecutive seasons. He observed that while there was no difference in the magnitude of the respiration rate at the climacteric maximum, there was some tendency for the peak to be attained earlier in light crop fruit. The higher values sometimes observed in the present investigations for the respiration rate in light crop fruit may merely arise from having measured the rate at a point in time when the fruit had reached a higher point on the climacteric rise. However, in cases where the preclimacteric respiration rate was higher in light crop fruit, a higher rate was also observed when measurements were made well on in the post-climacteric period. No explanation can be offered for this observation, and definite conclusions regarding respiration rate differences between light and heavy crop fruit must await more extensive investigations.

Internal carbon dioxide

In Cox apples in 1960 the light crop fruit was 23% lower in internal carbon dioxide level than the heavy crop fruit. This represented a difference in actual concentration of about 1.7%. However, the difference in the following year was in the reverse direction.

In Jonathan apples in 1960 the internal atmosphere concentrations were very low, and no difference was observed between light and heavy crop fruit. However, in the

following year the level of internal carbon dioxide was lower in light than in heavy crop fruit.

The initial stimulus to the investigations reported here was the possibility that the higher susceptibility of light crop fruit to low temperature breakdown might be due to a higher internal carbon dioxide concentration. This possibility was based on the fact that susceptibility to breakdown is greater in storage atmospheres containing carbon than in those without and also on the observation that internal carbon dioxide concentration varies directly with the external concentration. That light crop fruit in Cox in 1960, and in Jonathan in 1961, had a lower internal carbon dioxide concentration than heavy crop fruit in the same size groups disposes of this hypothesis associating higher susceptibility to breakdown in light crop fruit with higher internal carbon dioxide levels.

Further evidence on this point may be drawn from a comparison between the Cox fruit of three consecutive seasons.

In 1960 when no breakdown developed, internal carbon dioxide level was highest (5.6% in light crop, 7.3% in heavy crop) than in 1961 (4.7% in light crop, 3.7% in heavy crop) when two light crop fruits developed breakdown. These two fruits had a high internal carbon

dioxide concentration. The disorder was probably already developing at the time of the internal atmosphere measurements. Studies continued in the 1962 season are not yet complete and are not mentioned elsewhere in this thesis. In this season internal carbon dioxide levels were intermediate between those in the two previous seasons (5.1% in light crop 5.0% in heavy crop), while breakdown developed in 80% of the fruit. Of eight groups of ten fruits in this year not a single group showed a significant positive correlation between internal carbon dioxide concentration and breakdown incidence.

It appears that another explanation not based on a difference in internal carbon dioxide levels, must be found for higher susceptibility to breakdown in the light crop fruit.

Dry matter, soluble solids and free acids

In both years and in both varieties light crop fruit had a higher content of dry matter, soluble solids and free acids than heavy crop fruit. This may be explained on the basis of the higher leaf : fruit ratio obtaining in light crops. The dry matter consists largely of soluble solids, which in turn consist mainly of sugars derived from photosynthesis in the leaves. These sugars constitute the raw material for organic acid

production. With the higher leaf : fruit ratio in light crop trees carbohydrate raw material is supplied to individual fruits from a greater number of leaves and therefore in greater amount.

Nitrogen.

In Cox in 1960 total nitrogen content was lower in light than in heavy crop fruit, while in the following year the reverse was true. There was no difference in total nitrogen between heavy and light crop Jonathan apples in 1960, but in 1961 total nitrogen was lower in the light crop fruit.

The tendency for light crop fruit to be lower in total nitrogen content is commonly observed, and may be explained on the basis of competition between various tree parts for available nitrogen. The heavy yield of fruit in the season preceding the bearing of a light crop may deplete the nitrogen reserves of the tree to such an extent that the amount of nitrogen available to the light crop fruit is less than that available to the fruit in the previous year. During the growing season competition occurs for available nitrogen between fruits, leaves, and the following season's buds. Not only is the nitrogen content higher in the leaves and buds than in the fruits, but there are also more leaves and buds per

fruit in a light crop year. The increased competition may thus result in a lower nitrogen content in the fruit in the light crop year.

The higher nitrogen content in light crop Cox fruit in 1961 may be due to the carry over of a very large nitrogen reserve in this tree from the previous season.

Protein nitrogen contents showed the same differences as those found in total nitrogen. Since the contents of protein and total nitrogen are closely correlated in apple fruits, the same explanation applies to these differences between light and heavy crops as to the differences in total nitrogen.

Bitter pit

Bitter pit occurred only in the 1960 Cox fruit. Incidence was higher in light than in heavy crop fruit. This confirms the findings of Martin (1953, 1954), who demonstrated that bitter pit incidence was correlated negatively with crop size, as well as positively with fruit size both within and between trees. The present findings may also be linked with those of Smock (1937) who associated the incidence of bitter pit with the competition between leaves and fruits for water.

Jonathan spot

The incidence of Jonathan spot was lower in light than in heavy crop fruit in both years. This is at variance with the recent findings of Martin, Lewis and Cerny (1961) who have demonstrated a positive correlation between Jonathan spot incidence and fruit size, and a negative correlation between incidence and crop size. Results which are in accord with the findings of these authors were obtained when that fruit from the experimental trees which was not used in the main part of this study was subjected to normal cool storage. The data obtained on examination at the end of the storage period are shown in Table 6.

TABLE 6

		Jonathan spot %			
		Light	Medium	Heavy	Total
1960	Light	46.8	44.8	5.2	96.8
	Heavy	14.4	4.5	3.5	22.4
1961	Light	51.0	13.3	0.0	64.3
	Heavy	13.6	2.7	0.5	16.8

The difference in the behaviour of the 32 fruits in each year from that of the larger samples may perhaps be explained on the basis of the long delay before the samples were placed in cool storage, or alternatively of the injury inflicted on the fruit in the insertion of the gas pipettes.

TABLE 7

Correlations between variables : Levels of significance

		Cox		Jonathan	
	Year	Light	Heavy	Light	Heavy
Bitter pit - respiration rate	1960	xxx			
Bitter pit - nitrogen content	1960	xxx			
Jonathan spot - respiration rate	1960			xxx	xx
Protein nitrogen - respiration rate	1960	xxx		xxx	
	1961		xxx		x
Internal CO_2 - respiration rate	1960	xx			
	1961		xxx		
Protein nitrogen - internal CO_2	1960	x		xxx	
	1961	xxx	xxx		
Dry matter - soluble solids	1960	xxx	xxx	xxx	xxx
	1961			xxx	xxx
Dry matter - free acids	1960	xx			
	1961			xx	
Dry matter - colour	1960	xxx			
Colour-cell volume	1960	xx	xxx		
Jonathan spot - free acids	1960			x	xxx
Jonathan spot - soluble solids	1961			xxx	

x P 0.05

xx P 0.02

xxx P 0.01

Correlations between variables

Levels of significance are given in Table 7

(1) Bitter pit and respiration rate

In Cox in 1960 a positive correlation was found between bitter pit incidence and respiration rate in the light crop fruit only (See Figure 3). While there was no significant difference in respiration rate in 1960 between light and heavy crop fruit, the incidence of bitter pit was higher in the light crop fruit. In 1961 the light crop fruit had a higher respiration rate, but bitter pit did not occur. If higher respiration rate were solely responsible for higher incidence of bitter pit, then no difference would have been expected in the incidence of bitter pit between light and heavy crop fruit in 1960. It may be important to keep in mind that the respiration rate was measured immediately after picking, which was a long time before the disorder had developed. The positive correlation found in the light crop fruit in 1960 suggests that fruit with higher respiration rate, i.e. with a more rapid metabolism, will develop bitter pit in the presence of other hitherto unknown pit-inducing factors, and that these factors are more present in light crop fruit.

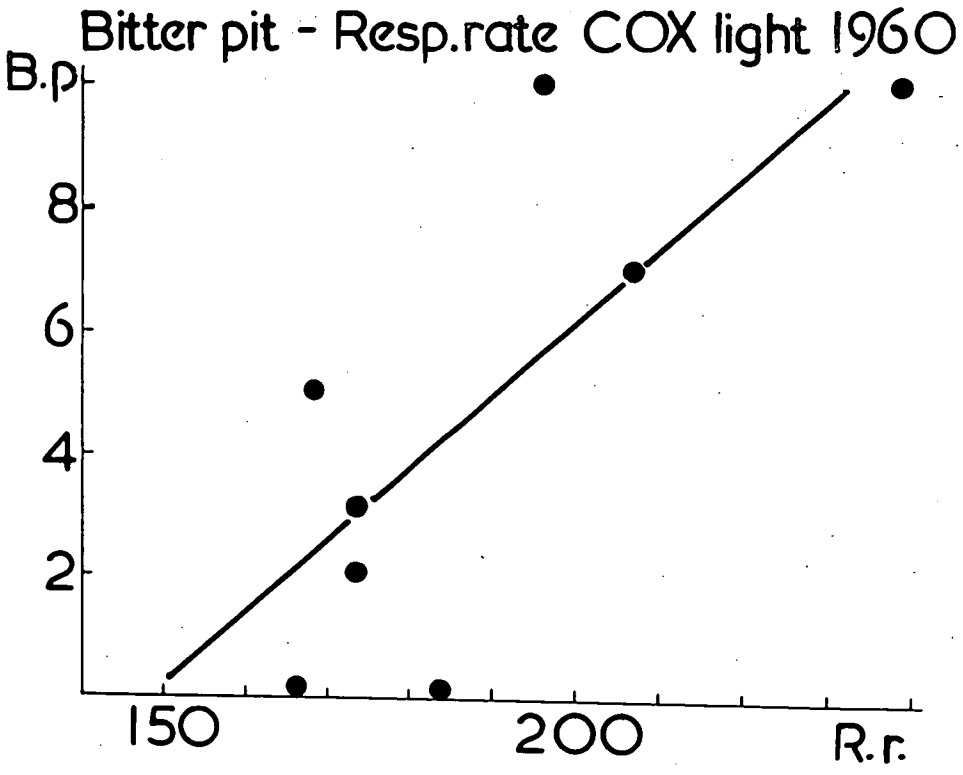

Figure 3

(2) Bitter pit and nitrogen content

Bitter pit incidence was positively correlated with total and protein nitrogen content only in the light crop Cox fruit in 1960, i.e. in the fruit in which incidence of this disorder was correlated with respiration rate (See Figures 4 and 5). Although the light crop fruit had lower contents of total and protein nitrogen, it had a higher incidence of bitter pit. Smock (1941) was able to increase the incidence of bitter pit with heavy nitrogen dressings and injections of urea into limbs, following the June drop. It must be assumed that, as in the case of respiration rate, higher nitrogen content may be partly responsible for an increase in the incidence of the disorder in the presence of other pit-inducing factors, especially in light crop fruit.

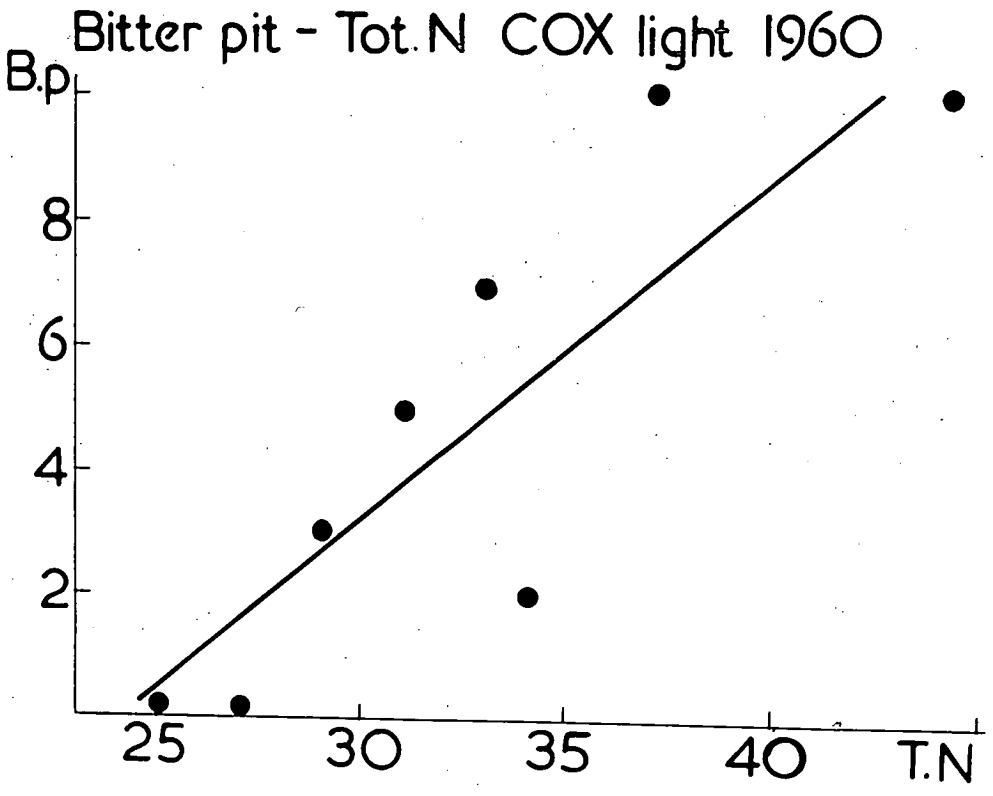

Figure 4

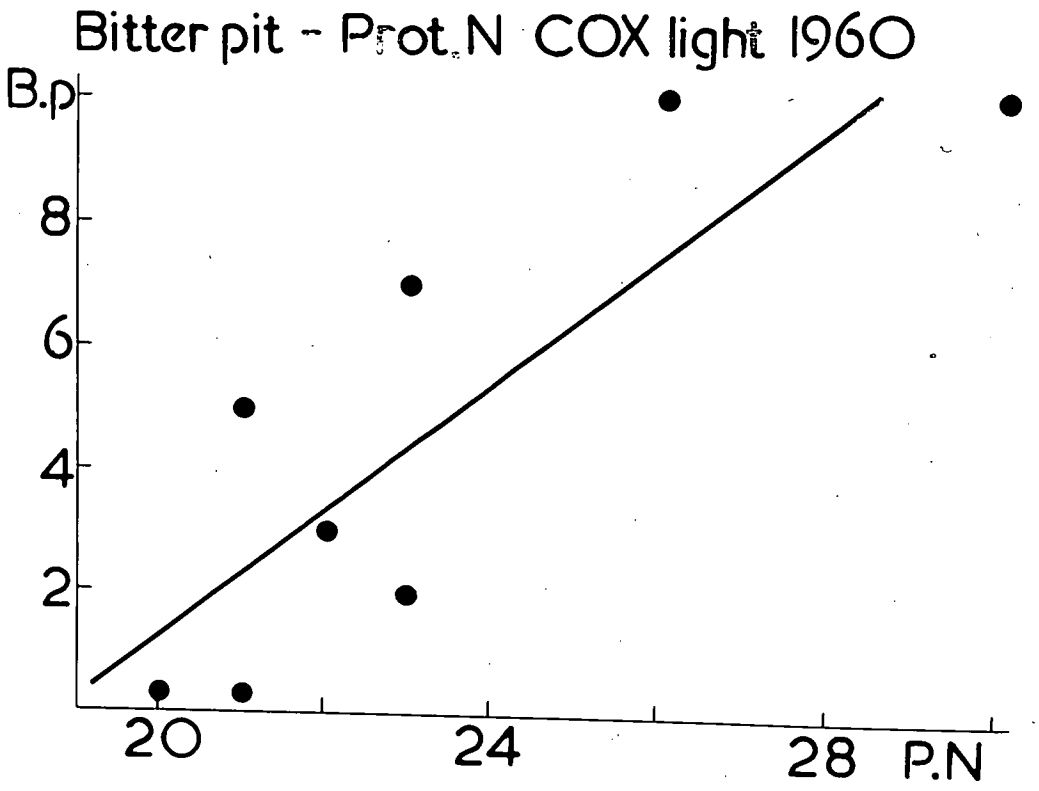

Figure 5

(3) Jonathan spot and respiration rate

Jonathan spot incidence was positively correlated with respiration rate in the 1960 season in both heavy and light crop fruit (See Figures 6 and 7). This suggests that high respiration rate may be at least partly responsible for the incidence and severity of Jonathan spot. If the apples are not cool stored the respiration rate at room temperature remains high, and the disorder appears within six to eight weeks. On the other hand, if fruit is placed in cool storage immediately after harvest, the respiration rate is maintained at a low level and the disorder does not appear until after three to four months. After removal of the fruit from cool storage the respiration rate increases with increase in fruit temperature, and the severity of the disorder increases simultaneously.

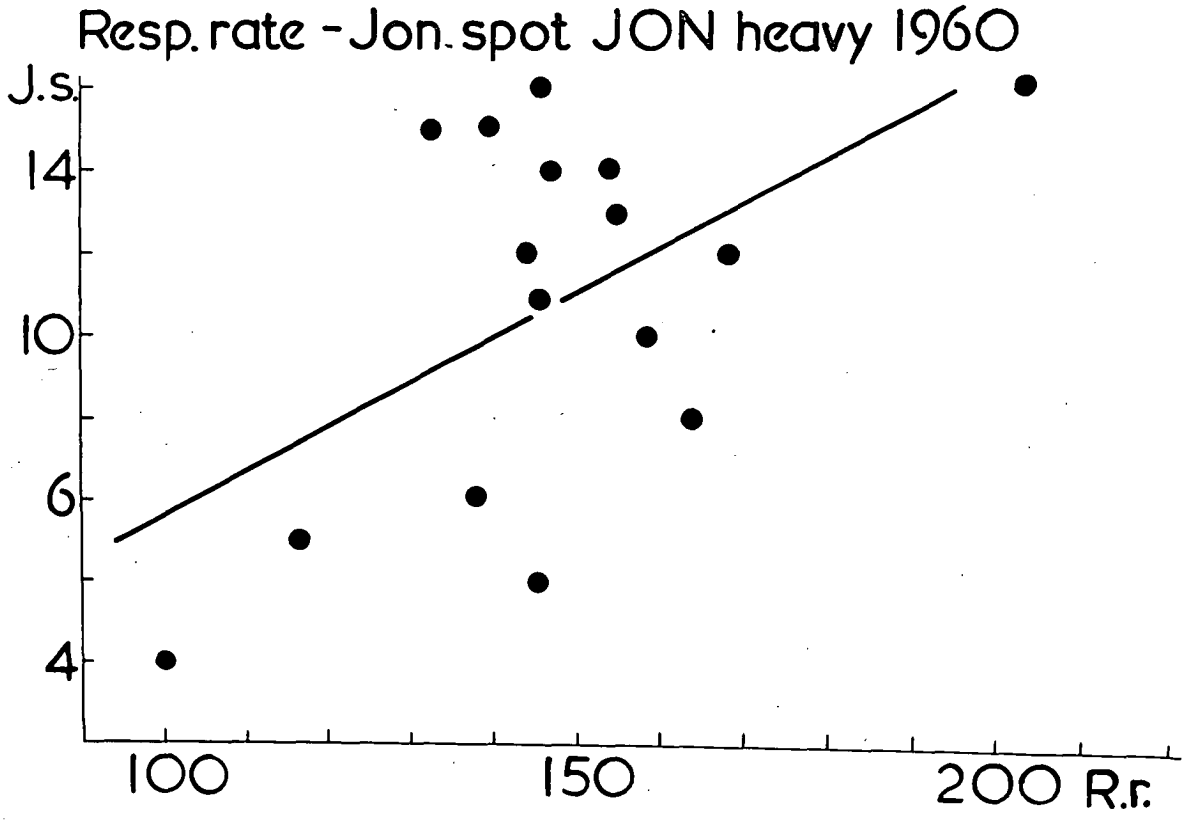

Figure 6

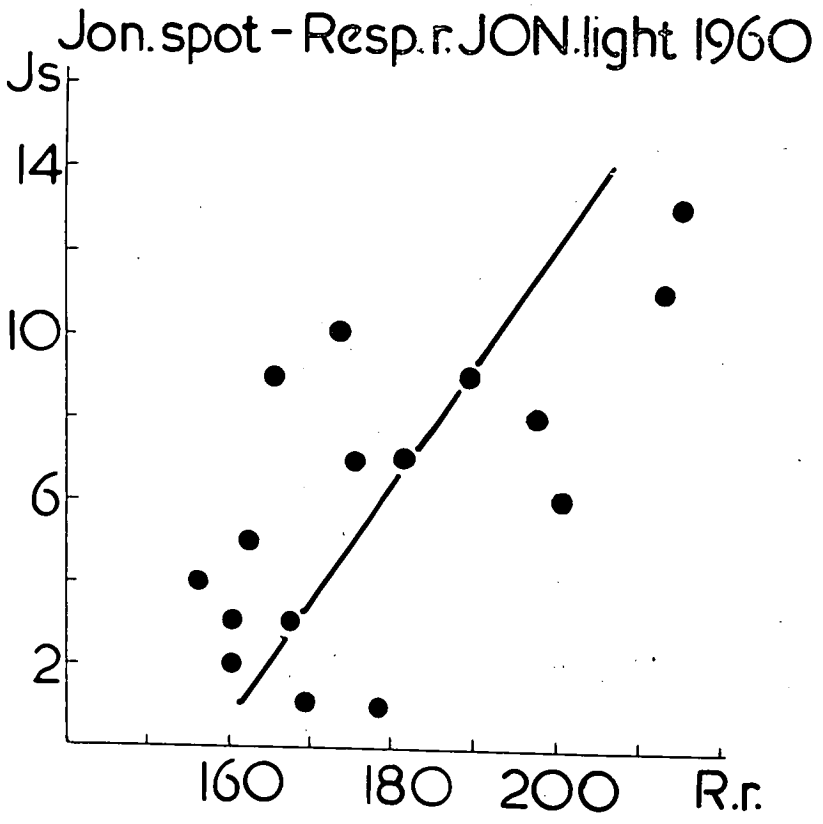

Figure 7

(4) Protein nitrogen and respiration rate

In both varieties these two variables were correlated positively in light crop fruit in 1960 and in heavy crop fruit in 1961. One of these correlations is illustrated in Figure 8. This is in agreement with the findings of Robertson and Turner (1951), who considered that respiration rate might be expected to increase with the protein nitrogen content, since an increase in the latter represents an increase in both total enzymes, including those of respiration, and other protoplasmic constituents requiring a constant supply of energy from respiration for their maintenance.

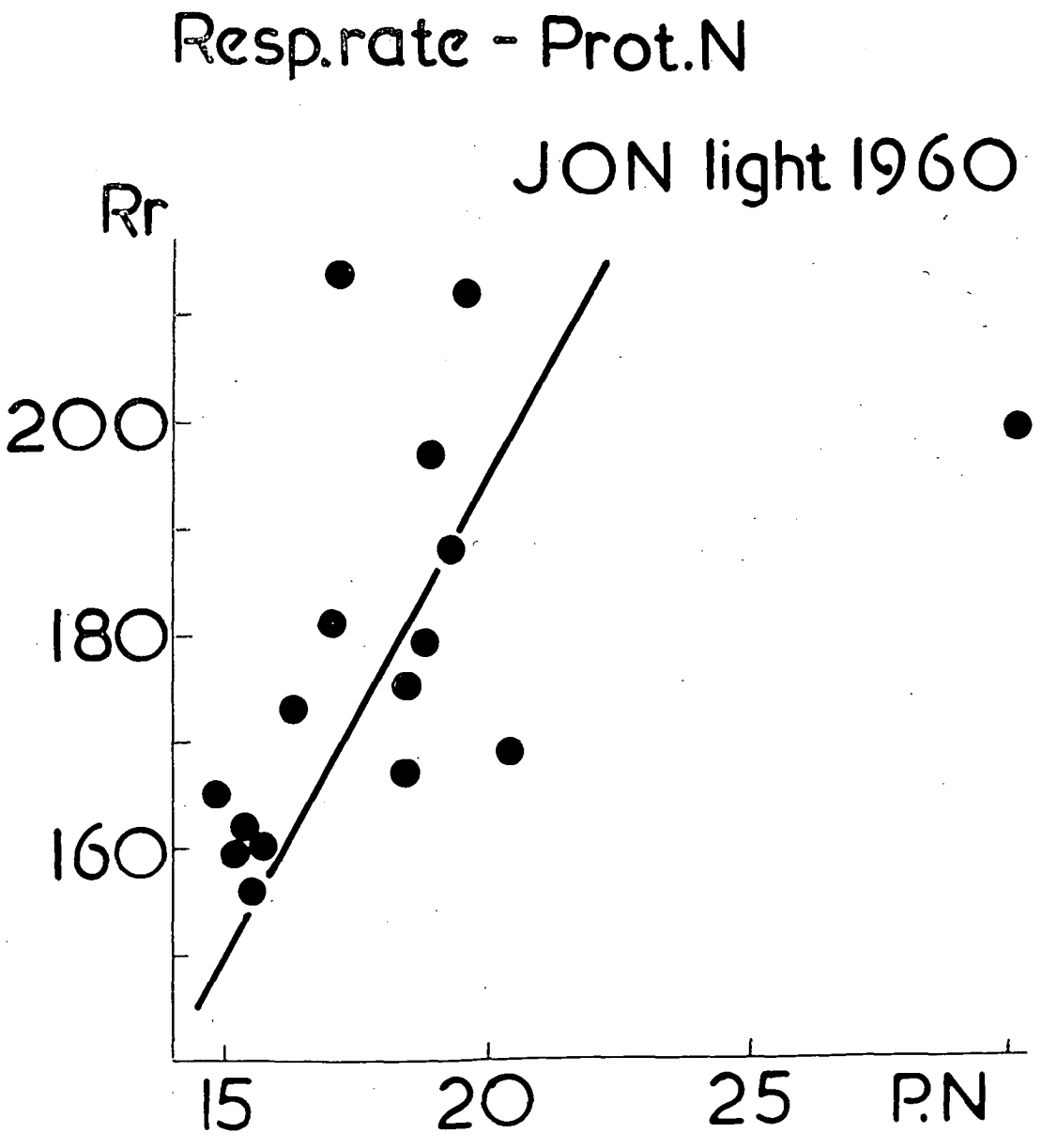

Figure 8

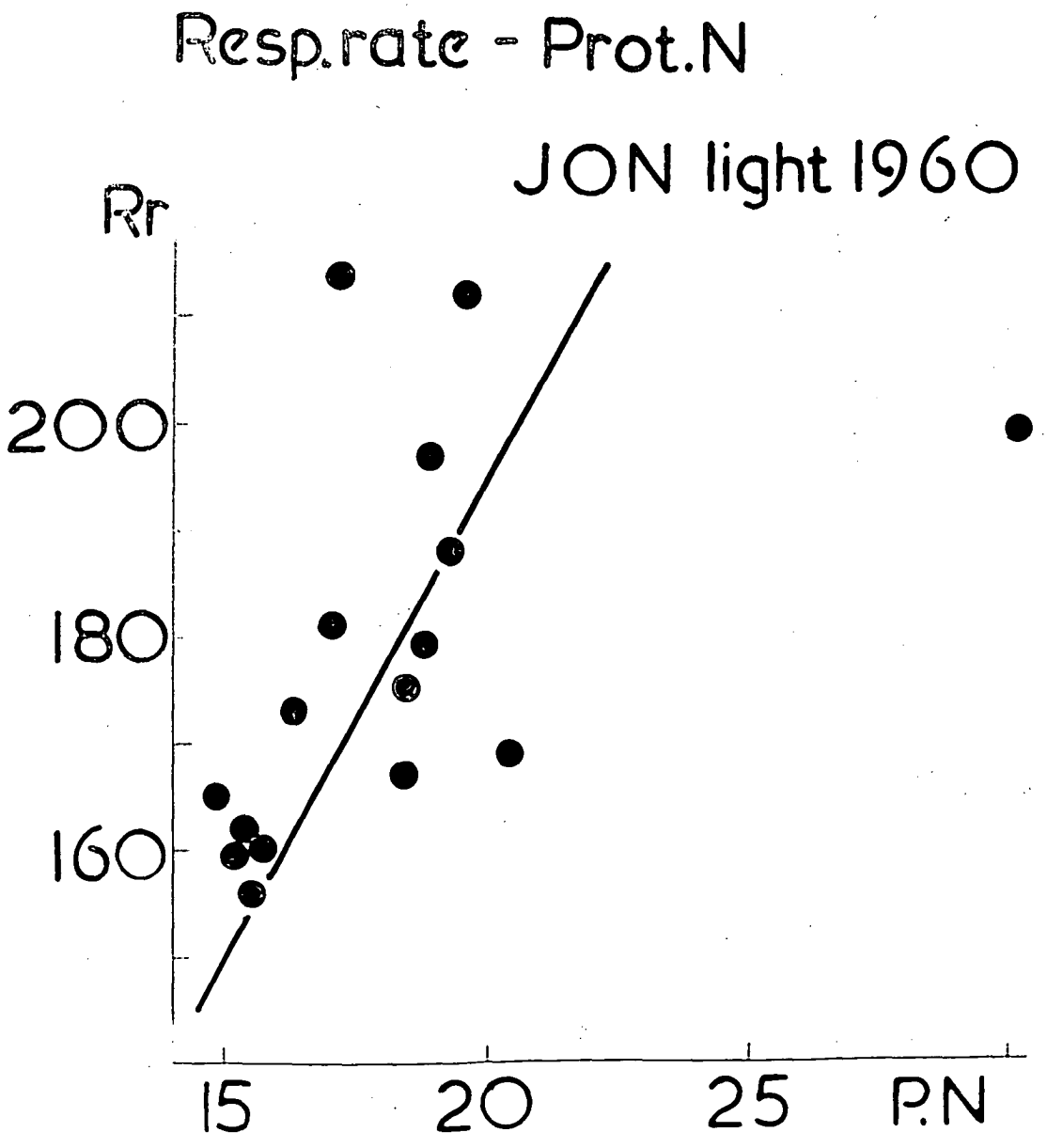

Figure 8

(5) Internal carbon dioxide and respiration rate

In the Cox variety there was a positive correlation between these variables in light crop fruit in 1960 and in heavy crop fruit in 1961. The latter correlation is illustrated in Figure 9. It has been stated earlier that in Cox in 1960 the internal carbon dioxide concentration was lower in light crop than in heavy crop fruit. At the same time there was no difference in respiration rate. On the other hand, while respiration rate in Jonathan in 1960 was greater in light crop than in heavy crop fruit, there was no difference in the level of internal carbon dioxide. These findings suggest that the skin of light crop fruit has a higher permeability to carbon dioxide.

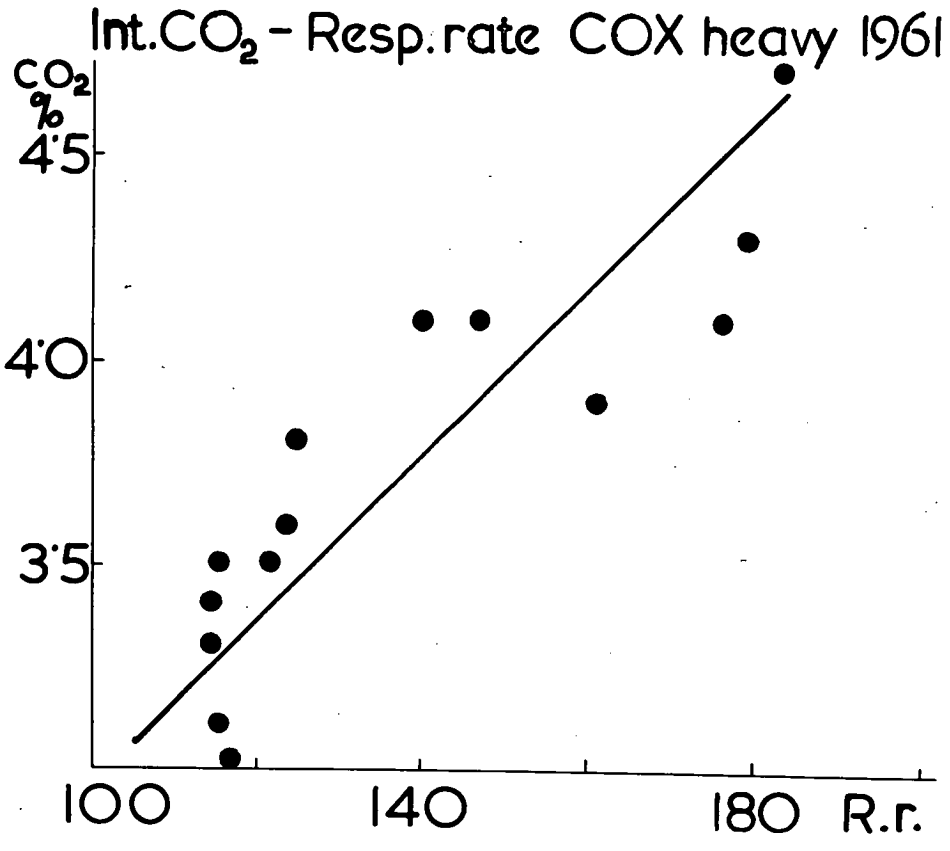

Figure 9

(6) Protein nitrogen and internal carbon dioxide

In the Cox variety these variables were positively correlated in light crop fruit in 1960, and in both heavy and light crop fruit in 1961. (See Figure 10). In the Jonathan variety this correlation was significant only in light crop fruit in 1960. Such a relationship would be expected in the light of the correlations found between respiration rate and protein nitrogen content, and between respiration rate and internal carbon dioxide concentration.

(7) Dry matter and soluble solids

In 1960 in both varieties a positive correlation was found between the percentages of dry matter and soluble solids. In the following year the correlation was significant only in Jonathan. This correlation is expected since soluble solids constitute a major fraction of the total dry matter in the fruit.

(8) Dry matter and free acids

In light crop fruit a positive correlation was found between these variables in Cox in 1960 and in Jonathan in 1961. This correlation is not unexpected since a major fraction of the dry matter is derived from photosynthesis, and the free acids constitute a part of this fraction. Both dry matter and free acids have been

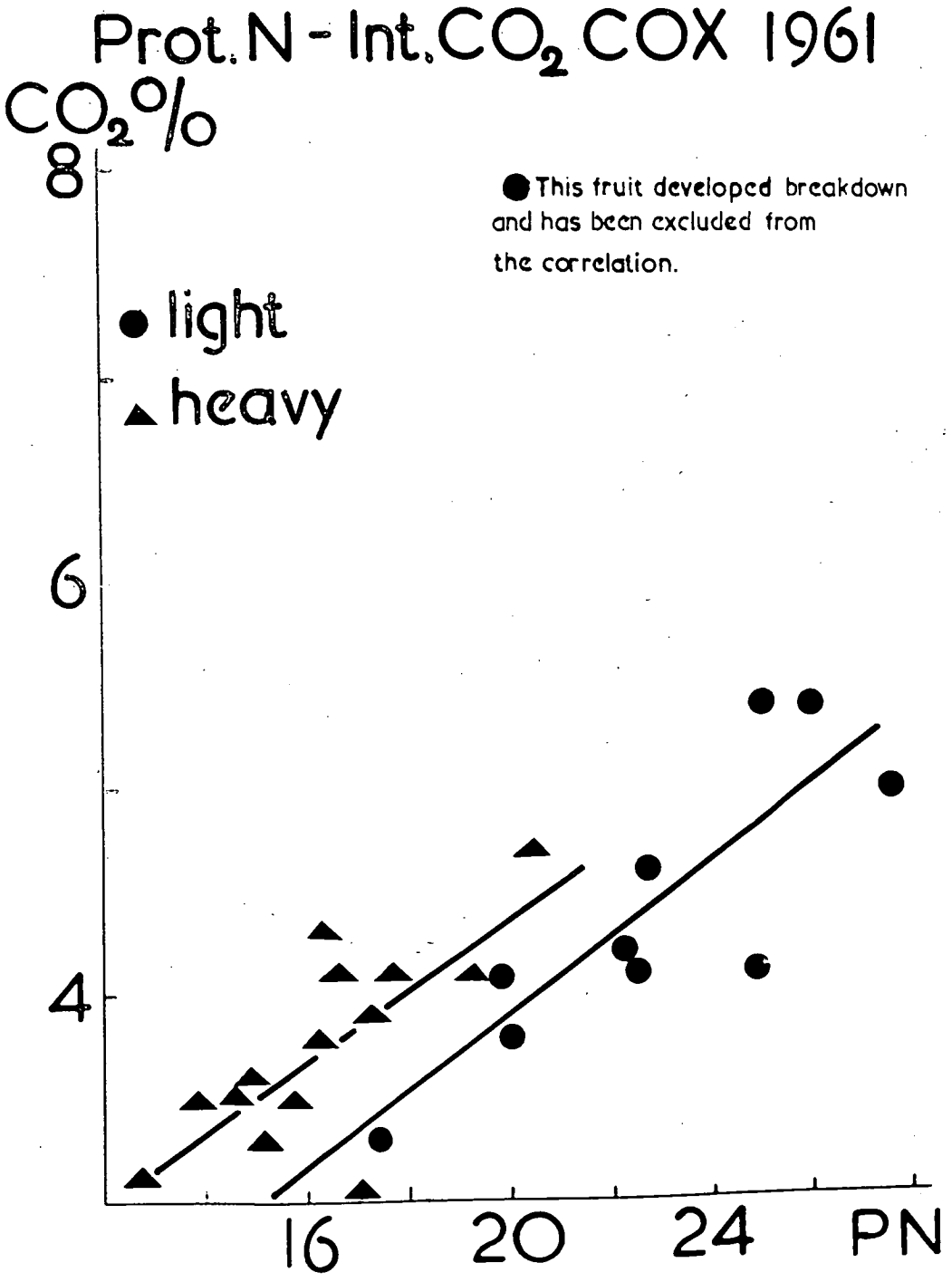
Prot. N - Int. CO_2 COX 1961
CO_2 %
8
6
4
● This fruit developed breakdown and has been excluded from the correlation.
● light
▲ heavy
16
20
24
PN

Figure 10

shown to increase during the growing period (Robertson and Turner 1951).

Martin (1954) has shown that total acid per fruit of a single tree may continue to rise right up to late maturity in some seasons, while in others it may fall continuously, and in others still it may rise and then fall. This variation from season to season may explain the failure to find significant correlations between dry matter and free acids in every case.

(9) Dry matter and colour

In the light crop Cox fruit in 1960 a positive correlation was found between the percentage of dry matter and the extent of the change in the ground colour from green to yellow (See Figure 11). This correlation would be expected since an increase in the dry matter percentage during maturation of apples has been observed by Hopkins and Gourley (1933), by Askew (1935) and by Fraser (1951), and the extent of ground colour change is an indication of the degree of maturity.

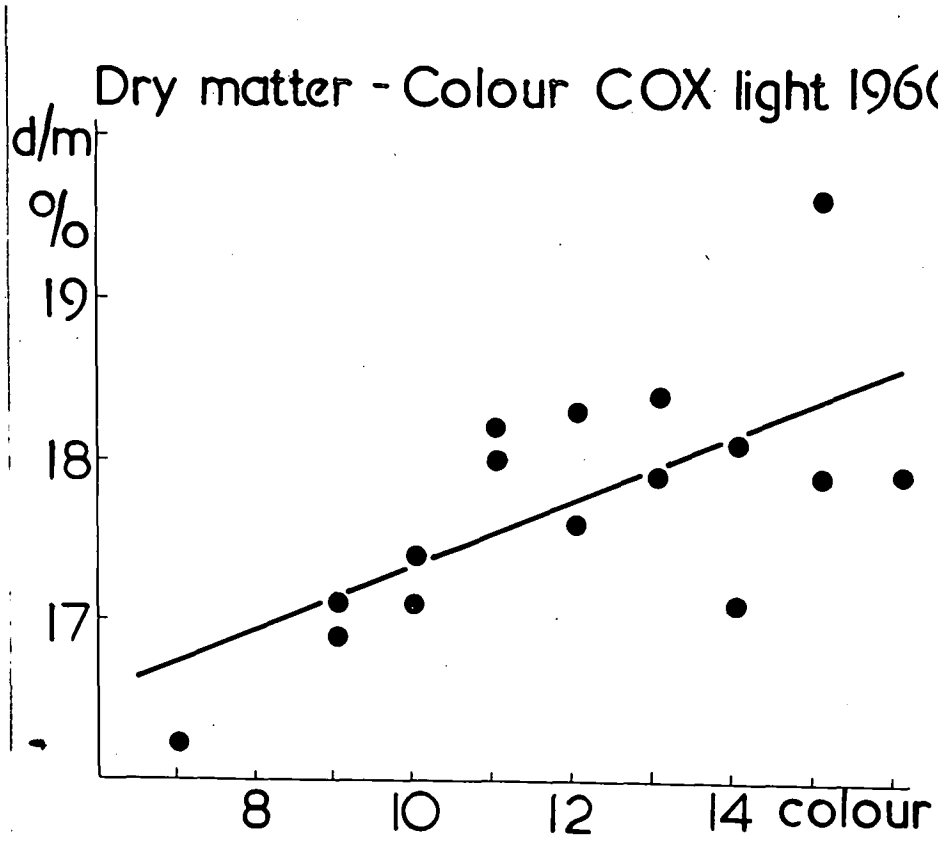

Figure 11

(10) Colour and internal carbon dioxide

In both years there was a negative but not significant correlation between the extent of ground colour change from green to yellow and the internal carbon dioxide concentration. The correlation was significant at the 1% level in separate samples of 50 fruits from each tree in 1960. The findings are in agreement with those of Trout et al. (1942) who demonstrated that degree of colour change was correlated positively with internal oxygen concentration and negatively with internal carbon dioxide concentration. The greener colour normally observed in skin-coated fruit and in fruit stored in an atmosphere high in carbon dioxide is also in accord with the present observations.

(11) Colour and cell volume

In 1960 a positive correlation was found between the extent of ground colour change from green to yellow and the cell volume, in both light crop and heavy crop Cox fruit (See Figure 12). This means that the fruit with the highest rate of cell expansion also has the highest rate of colour change, suggesting that there is a strong association between the processes of cell expansion and senescence.

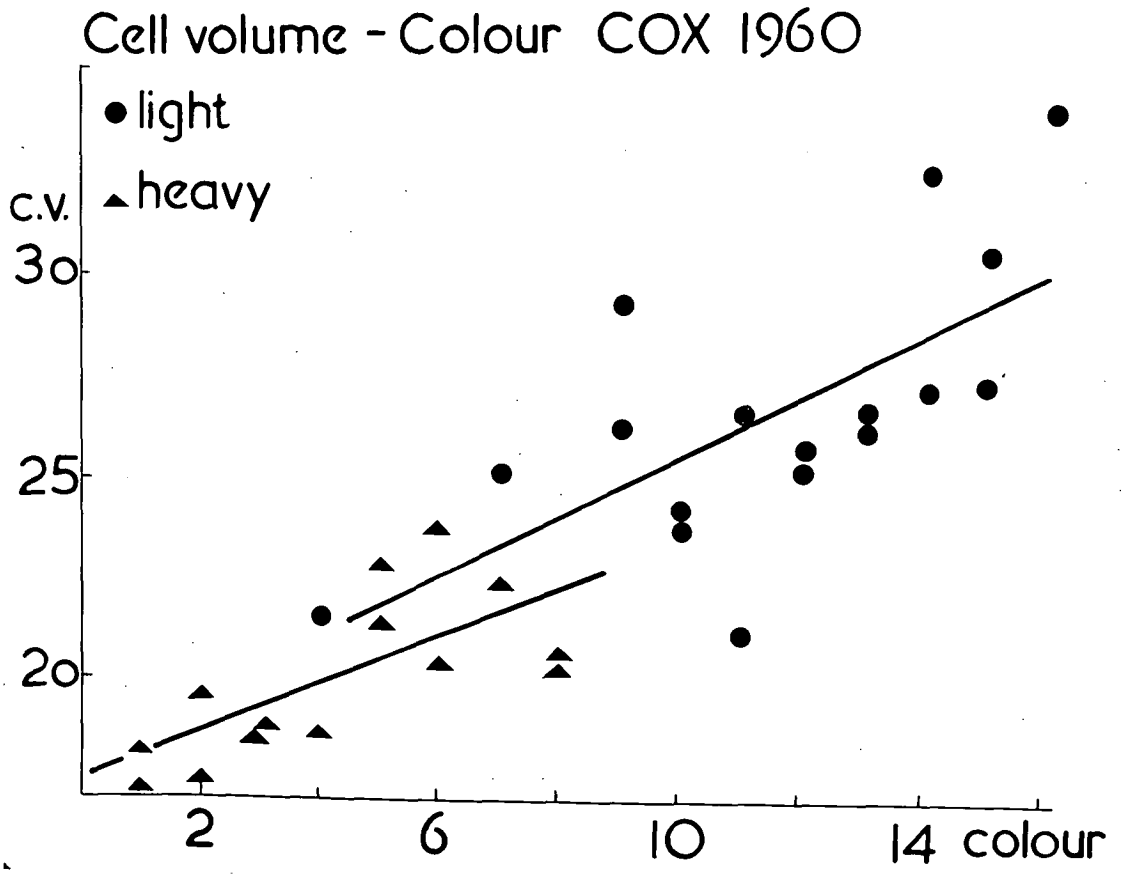
Cell volume - Colour COX 1960
● light
▲ heavy
c.v.
30
25
20
2
6
10
14 colour

Figure 12

(12) Jonathan spot and soluble solids

These two variables were positively correlated in light crop fruit in 1961, as shown in Figure 13. This may be explained as a maturity effect. The more advanced the maturity of the fruit at time of harvest, the greater will be both the level of soluble solids and the susceptibility to Jonathan spot. This finding is linked with that of Martin et al.(1961) who reported that light crop fruit, which had a higher soluble solids content than heavy crop fruit, also had a higher susceptibility to Jonathan spot.

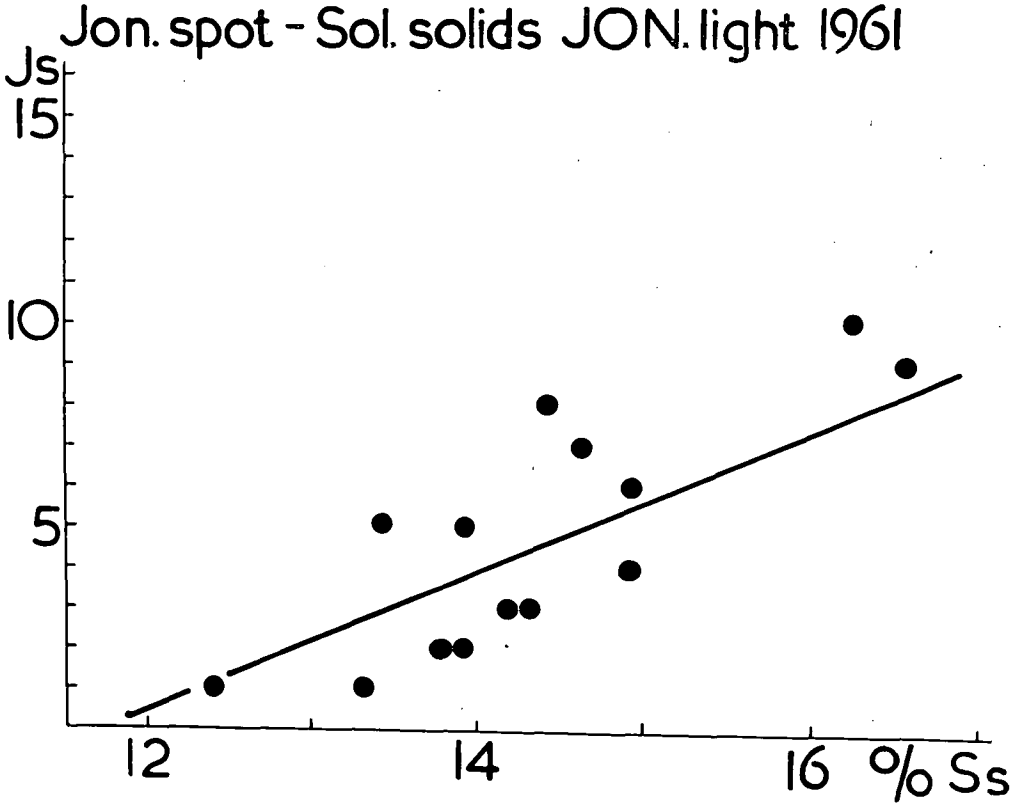

Figure 13

(13) Jonathan spot and free acids

In 1960 there was a negative correlation between Jonathan spot incidence and the percentage of free acids in both light and heavy crop fruit. This correlation may also be explained as a maturity effect, since the actual concentration of free acids in the tissue declines during maturation, and more mature fruit is more susceptible to Jonathan spot. This observation is in accord with the findings of Plagge and Gerhardt (1930) that Jonathan fruit with higher acidity was less liable to Jonathan spot than fruit in which free acids content was allowed to diminish by delaying cool storage.

CONCLUSIONS

Investigations have been carried out, in the two successive seasons 1960 and 1961, on individual apple fruits of the varieties Cox's Orange Pippin and Jonathan. With each variety fruits were taken from two trees of contrasting cropping level. From each tree eight apples in the 2¼" size group and eight in the 2½" size group were selected so that for each fruit from a light crop tree there was a corresponding fruit of equal weight from the heavy crop tree. Certain measurements and analyses were made on the individual fruits, and the results have been treated statistically. Some of the more important findings and conclusions derived from them are set out below.

The presence of only partly developed seeds evenly spaced in the carpel suffices to ensure the development of a fruit of perfect shape and normal size.

Light crop fruit was higher in dry matter, soluble solids and free acids, and had a yellower ground colour, than heavy crop fruit. This was attributed to the larger number of leaves present per fruit on the lightly bearing trees.

The concentration of carbon dioxide in the internal atmosphere was higher in heavy crop fruit in Cox in 1960 and in Jonathan in 1961. This has been

explained on the basis of a postulated higher resistance in heavy crop fruit to gaseous diffusion, probably located in the skin. This finding disposes of the suggestion that the higher susceptibility to low temperature breakdown which is characteristic of light crop fruit might be due to higher internal carbon dioxide levels.

The higher respiration rates observed in light crop fruit after harvest in Jonathan in 1960 and in Cox in 1961 are associated with lack of difference in cell volume and in internal carbon dioxide level. At present no explanation can be suggested for these findings. The finding of Hackney (1943) that respiration rate is governed by internal oxygen concentration was not supported by the observed differences between light and heavy crop fruit in respiration rate and internal oxygen level. Further investigation into this question is needed.

The lower nitrogen content observed in light crop fruit has been attributed to the greater competition from the leaves and buds on a lightly bearing tree, and to the depleting effect of the heavy crop in the previous year.

The higher cell number per fruit found in heavy crop fruit in most cases may have resulted from a higher rate of cell division, or a longer period of cell division,

or a combination of both, due to a more plentiful supply of the factors necessary for cell division.

The greater susceptibility of light crop fruit to physiological disorders, as observed in these studies, is in agreement with the findings of Martin and Lewis (1952) from work with a number of apple varieties. The findings from much of the present work tend to support the view that maturation and senescence in light crop fruit proceed more rapidly than in heavy crop fruit, and consequently the life span of the fruit is shorter. It is possible that light crop fruit should be harvested earlier than heavy crop fruit for comparable keeping quality, and further studies directed towards this problem may be profitable.

The positive correlations observed within a tree between dry matter and colour, soluble solids and free acids, and cell size and colour, suggest that each fruit is an individual with its own rate of growth and maturation, and that this rate is conferred upon the fruit by the bud rather than by the tree as a whole.

In the present studies the factors which appear to be most closely linked with the incidence of the disorders bitter pit and Jonathan spot are crop size, fruit size, cell size, respiration rate and nitrogen level. The negative correlation between Jonathan spot incidence and free acids content may be explained as a maturity effect.

From a practical point of view, evaluation at harvest of the average cropping level and fruit size for a group of trees of the same variety would give the producer an indication of the potential storage life of the fruit. Cell size and respiration rate measurements are tools which could be used by the breeder in searching for new strains and varieties characterized by improved keeping quality. The nutritionist and plant physiologist wishing to improve keeping quality in varieties already established commercially may profit by looking for substances which increase the number of cells per fruit and decrease cell size and respiration rate.

The number of significant correlations observed in this work has justified the study in basic research of individual apples from a single tree. The method has the advantage of economy of time, labour and materials.

Further investigations in this field will be extended to include a determination of the resistance to gaseous exchange and its location in the fruit, and a study of the possible roles of the mineral elements calcium, potassium, magnesium and phosphorus in the incidence of bitter pit.

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THE PHYSIOLOGY OF GROWTH IN APPLE FRUITS

VII. BETWEEN-TREE VARIATION IN CELL PHYSIOLOGY IN RELATION TO DISORDER INCIDENCE

By D. MARTIN, T. L. LEWIS, and J. CERNY

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THE PHYSIOLOGY OF GROWTH IN APPLE FRUITS

VII. BETWEEN-TREE VARIATION OF CELL PHYSIOLOGY IN RELATION TO DISORDER INCIDENCE

By D. MARTIN,* T. L. LEWIS,* and J. CERNY*

[*Manuscript received March* 25, 1954]

Summary

Mean cortical cell size, soluble and protein nitrogen per cell, preclimacteric respiration, mean fruit size, and incidence of disorders have been studied for fruit of each tree in a plot of 35 trees of Jonathan variety. These trees were remarkably uniform with regard to soil, aspect, tree size, and pollinating variety, but provided a range of mean fruit size per tree.

There was a high degree of correlation between the variables. Cortical cell size increased with mean fruit size but more rapidly than would be expected from a proportional increase with size of fruit. Protein nitrogen increased proportionally with cell volume but the ratio of protein nitrogen and cell surface increased with cell size, suggesting that the protoplasm increased in thickness with cell size. Intercorrelation between respiration per cell, protein nitrogen, soluble nitrogen, and cell size were particularly close, remaining highly significant even when mean fruit size per tree was held constant by methods of partial correlation analysis, suggesting that these characteristics are functions of cell growth and are not influenced by between-tree differences due to cropping. Disorder incidence is correlated with the other variables and the implications of these relationships are discussed.

I. INTRODUCTION

The keeping quality of fruit is determined while it is on the tree. Study of the between-tree variation in fruit physiology under uniform cultural conditions may thus be a useful approach to the improvement of keeping quality.

Earlier work (Martin 1954) showed that the best index of susceptibility to disorder in fruits from different trees was the mean fruit size per tree. This was better than any of the other indices associated with ripening (e.g. acid and soluble solids concentration, starch or ground colour change) and it was not improved when combined with any of these indices. Martin and Lewis (1952) showed that between varieties, cell volume, respiration per cell, protein nitrogen per cell, and respiration rate per unit protein (Hulme 1951) were positively correlated.

Robertson and Turner (1951) showed that, in fruit maturing on a single tree, protein synthesis kept pace with cell enlargement and they put forward the hypothesis that higher protein contents made greater demands on the energy distributors of the cells and resulted in higher respiration rates.

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These findings suggested that a study of one variety over a range of crop sizes might help in the elucidation of the remarkably close relation between mean fruit size per tree and breakdown incidence, and might be a useful introduction to attempts to improve keeping quality.

II. Material and Methods

In the 1951-52 season a block of Jonathan trees, 30 yr old and exceptionally uniform with regard to tree size, soil type, and slope became available. It consisted of six adjacent rows of trees planted 16 by 16 ft extending across the orchard with Cleopatra variety for the next six rows adjacent. The mean crop per tree over 4 yr ranged from 600 to 1000 fruits. The trees received no manuring for the season 1950-51 and the 35 trees used for this study were not manured for the 1951-52 season.

On March 19, 1952 a sample of 20 fruits and another of 150-200 fruits were taken by a procedure designed to produce random sampling. The former was used to determine respiration rate at 25°C by a modification of the method of Eaves (1938), taking the mean rate for the 40-48 hr after picking as did Hulme (1951). This respiration was preclimacteric. From 10 of these fruits a transverse section was cut from the mid-cortex region of each fruit midway between stem and calyx, fixed in formalin-acetic-alcohol, stained with ruthenium red, and mounted in "Euparal." Mean cell size was determined on the basis of the work of Bain and Robertson (1951) and by a sampling method of cell ranking devised by McIntyre (1953). This method was about twice as efficient as random sampling for the same number of cells measured. The mid-cortical tissue of the 20 fruits was sliced and dried at 65°F, powdered, and stored in sealed jars at 32°F for protein analysis by the methods used previously (Martin and Lewis 1952).

The larger samples of 150-200 fruits were stored at 33-34°F for 7 months, when they were removed to room temperature. Mean fruit size was then determined and the fruits were examined for disorders immediately, and again after 2 wk at room temperature.

The use of samples of differing sizes introduces difficulties in the mathematical treatment which will be referred to later. Mean fruit size and disorder incidence are easily determined and on a sample of 150-200 fruits involve sampling standard errors relative to the mean of only ± 1 per cent. in mean fruit size and ± 3 per cent. in disorder.

Respiration rate and protein and soluble nitrogen contents are determined on samples of only 20 fruits; from a limited number of duplicates it is estimated that the coefficient of variation for bulked material from 20 fruits is of the order of 5·5 per cent.

Determinations of mean cell size from two samples of 10 fruits gave results which differed by 1 per cent. The sampling error in the determination of mean cortical cell size is of the order of ± 5 per cent. Mean cell size can be regarded in two ways: (*a*) as an estimate of mean cell size of the cortical tissue; and (*b*) as an estimate of mean cell size for the whole fruit. This esti-

mate of mean cell size for the whole fruit is based on the work of Bain and Robertson (1951) and the validity of a comparison between trees requires the assumption that the cell size gradient within fruits did not differ between trees. As the range of mean fruit size between trees in this plot was not great (83-113 g), we consider this assumption justified, and a comparison of mean cell number per fruit to be possible by their methods.

Respiration rate cannot be determined from the cortical tissues only. However, the rate for the whole fruit cannot be very different from that for the cortical tissues only as the cortex is approximately 80 per cent. of the total respiring tissue.

III. Results

The data for the 35 trees are set out in Table 1, and correlation coefficients for a number of variables are given in Table 2. Some of the correlations are examined further by methods of partial correlation in Table 3.

(*a*) *Disorder Incidence and Fruit Attributes*

(i) *Mean Fruit Size.*—The correlation of mean fruit size and percentage breakdown provides further evidence for the accuracy of mean fruit size per tree as an index of breakdown susceptibility in the fruit.

(ii) *Cell Size.*—The correlation of cell size and disorder incidence supports the suggestion in an earlier paper in this series (Martin and Lewis 1952) that variations in disorder level might be related to difficulties of cell organization associated with cell growth.

(iii) *Respiration per Unit Protein.*—This attribute was determined prior to storage, and disorder incidence was the result of 7 months storage, during which respiration proceeded at some function of the prestorage rate. The correlation of these two variables, which are separated by such a long time interval, heightens the possibility that disorder incidence might be determined by difficulties in protein maintenance; the differences in respiration rate per unit protein over the storage period determining the relative depletion of reserves and the extent of breakdown.

(*b*) *Relation of Rots Developing in Store to Mean Fruit Size*

Positive correlation occurred between percentage rots visible when the fruit was removed from store and mean fruit size; with further infections during the period at room temperature, the correlation declined to insignificance. The relationship has not been noted before and while not relevant to the main theme, is reported here as a factor which should be considered in all experiments on rotting where different populations of fruit are compared.

(*c*) *Interrelation of Other Fruit Attributes*

(i) *Mean Fruit Size, Cell Size, and Cell Number per Fruit.*—There was a high positive correlation between mean fruit size and mean cell size but there

TABLE I

FRUIT ATTRIBUTES OF PLOT OF 35 JONATHAN TREES

Mean Fresh Weight (g)	Disorder (%) = 100 − Sound	Breakdown (%)	Breakdown 2¼ in. Fruit (%)	Rots in Store (%)	Jonathan Spot (%)	Cell Volume (cu. mm. × 10^{-6})	Cell Surface (sq. cm. × 10^{-4})	Cell Number per Gram	Respiration per Cell (mg × 10^{-11} CO_2/hr)	Protein Nitrogen per Cell (g × 10^{-14})	Respiration/Unit Protein (mg × 10^{-3} CO_2/g protein N/hr)	Soluble N/Cell (g × 10^{-14})
1	2	3	4	5	6	7	8	9	10	11	12	13
83·7	14·9	10·9	14·0	0·9	17·2	180	9·67	505	463	415	112	177
88·3	34·3	23·7	23·0	4·0	15·2	176	9·60	515	433	389	120	125
88·1	20·3	2·7	3·0	2·2	18·5	148	8·77	622	448	335	134	96
88·0	32·2	13·2	12·0	4·0	19·5	130	8·15	700	380	320	121	94
90·8	45·4	27·6	49·0	5·4	24·9	151	8·94	602	510	357	143	145
91·5	39·5	15·7	29·0	2·3	36·5	183	9·89	496	523	419	125	155
92·0	42·5	26·4	36·0	1·1	25·3	171	9·46	532	500	383	130	98
93·4	39·9	25·8	32·0	1·2	21·5	155	8·99	586	538	365	148	124
93·4	46·8	20·7	34·0	1·8	36·7	171	9·45	582	532	363	147	113
95·0	60·5	59·7	64·0	1·8	14·6	130	8·20	700	381	300	127	112
95·2	37·5	14·7	19·0	7·6	25·9	181	9·73	503	548	403	137	214
95·2	43·0	32·3	43·0	2·5	19·0	164	9·31	553	374	374	118	90
97·5	48·4	38·5	50·0	3·7	14·8	154	8·99	590	485	394	123	128
97·7	53·8	2·8	31·0	3·9	44·9	200	10·27	455	830	475	171	195
97·7	51·5	34·6	41·0	5·0	25·8	180	9·71	505	659	441	150	163
98·0	56·5	39·5	47·0	6·2	18·1	186	9·77	489	694	442	157	228
98·7	58·6	39·4	56·0	2·6	27·7	179	9·68	507	612	392	156	140
100·6	49·0	35·6	41·0	5·0	15·9	187	9·98	485	627	440	143	148
100·7	55·8	32·8	35·0	3·4	44·8	220	10·90	413	852	498	171	309
101·3	62·3	46·3	58·0	3·2	27·8	221	11·07	412	771	520	148	260
101·2	58·1	42·7	48·0	8·0	13·2	216	10·69	420	746	494	151	181
101·9	60·2	24·7	32·0	4·2	46·4	168	9·40	540	501	355	142	165
102·5	74·4	49·7	56·0	4·8	44·9	203	10·44	448	772	460	168	146
102·9	57·4	26·9	29·0	2·4	38·9	174	9·46	522	597	417	143	142
103·5	73·1	44·9	51·0	7·8	41·6	174	9·50	522	672	452	147	191
103·5	51·8	36·0	45·0	10·8	7·9	225	10·44	403	833	552	151	298
105·0	51·9	35·0	43·0	7·3	21·2	196	10·10	463	666	490	136	235
104·3	56·0	40·5	40·0	10·0	13·5	202	10·20	450	765	483	150	195
104·5	68·0	54·6	54·0	5·3	17·3	215	10·85	422	690	485	143	153
105·8	63·6	53·7	58·0	4·6	13·0	231	11·23	394	882	939	164	305
107·7	89·0	78·6	83·0	6·2	29·0	263	12·13	396	980	600	160	291
108·4	75·3	46·6	59·0	6·0	32·7	224	10·93	406	855	482	172	216
109·7	90·1	68·3	71·0	5·0	46·7	256	11·81	355	1085	605	172	287
112·7	83·8	82·3	82·0	6·8	6·2	250	11·60	364	959	531	181	262
112·7	80·0	69·8	66·0	6·8	16·7	217	10·51	418	900	551	163	240

was a significant increase in the residual variation if the fitted regression line of cell size on fruit size was made to pass through the origin. The mean cell size in the cortical tissues increased more rapidly than would have been expected from a proportional increase with the size of the fruit. If the mean cortical cell size is taken as an estimate of the mean cell size of the whole fruit, the converse aspect of this relation is the negative correlation of the total cell number with fruit size (see Table 2). This implies the possibility that the stimulus to cell division may be weaker in light-crop fruitlets; light-crop trees may have not only fewer fruit buds but less cell division in the fruits although they are larger. This point is being investigated further.

TABLE 2
CORRELATIONS OF VARIABLES

Correlation	r	Significance
Mean fruit size and percentage disorder	0·9034	$P<0·001$
Mean fruit size and percentage breakdown (all fruit)	0·7928	$P<0·001$
Mean fruit size and percentage breakdown (2½ in. fruit)	0·6505	$P<0·001$
Mean fruit size and percentage Jonathan spot	0·1003	N.S.
Mean fruit size and percentage rots developed during cool storage	0·6157	$P<0·001$
Mean fruit size and percentage rots at final examination	0·2505	N.S.
Mean fruit size and mean cell volume	0·7613	$P<0·001$
Mean fruit size and mean cell surface	0·7373	$P<0·001$
Mean fruit size and cell number per fruit	−0·4452	$P<0·01$
Mean fruit size and respiration per cell	0·8320	$P<0·001$
Mean fruit size and protein N per cell	0·7822	$P<0·001$
Mean fruit size and soluble N per cell	0·6677	$P<0·001$
Mean fruit size and respiration/protein	0·7480	$P<0·001$
Mean cell surface and protein N per cell	0·9328	$P<0·001$
Protein N per cell and respiration per cell	0·9371	$P<0·001$
Protein N per cell and R/P ratio	0·6705	$P<0·001$
Protein N per cell and soluble N per cell	0·8661	$P<0·001$
Protein N per cell and percentage breakdown	0·7010	$P<0·001$
Percentage disorder and R/P ratio	0·7513	$P<0·001$
Percentage breakdown and mean cell volume	0·6416	$P<0·001$
Percentage breakdown and R/P ratio	0·5336	$P<0·001$
Percentage breakdown and percentage Jonathan spot	0·5020	$P<0·001$

(ii) *Mean Cell Volume, Cell Surface, and Protein Nitrogen.*—Over the range of mean cell sizes provided by these data the relation of cell volume to cell surface was linear (Fig. 1) owing to the tendency for the cells to increase faster along the major axis than along the minor axis.

The relation of protein nitrogen per cell to cell volume was particularly close, remaining highly significant when mean fruit size per tree was held constant. The protein nitrogen/cell volume line (Fig. 2) passed through the origin or very close to it, showing a proportionality between protein and cell volume.

The ratio of protein nitrogen to cell surface increased as cell surface increased (Fig. 3), suggesting either that the protoplasm of the cortical cells increased in thickness as cell size increased or that the protoplasm became more concentrated.

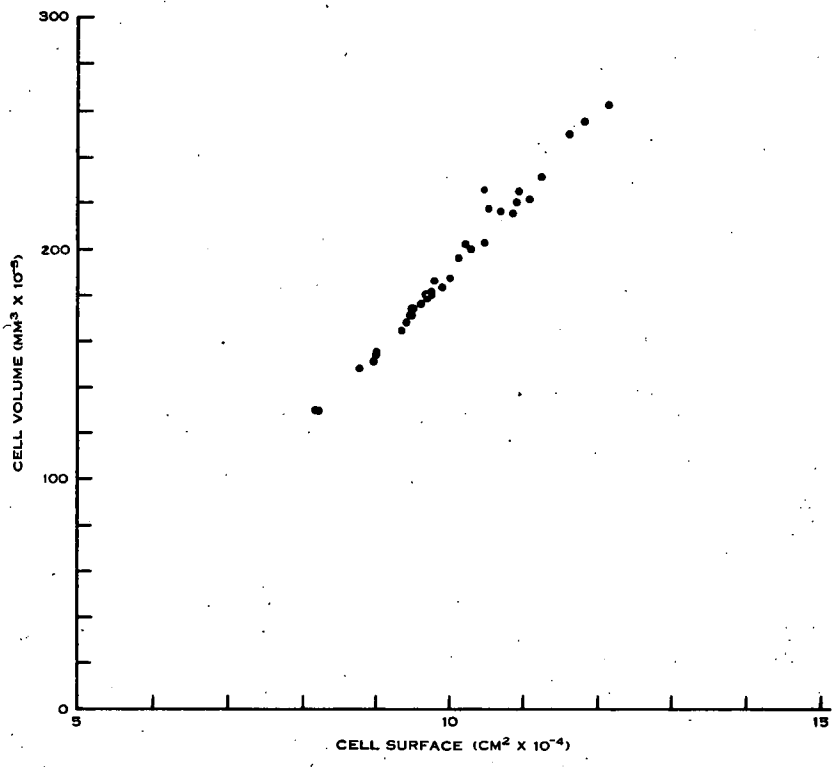

Fig. 1.—Cell surface and cell volume.

(iii) *Protein Nitrogen, Soluble Nitrogen, and Respiration.*—The close relation between protein nitrogen per cell and preclimacteric respiration per cell illustrates the interdependence of these two factors over a range of crop levels. Because the relation between cell volume and cell surface was linear over the range available (Fig. 1), soluble nitrogen was linearly related to both cell volume and cell surface (Figs. 2 and 3). Thus no information was available to suggest whether soluble nitrogen was contained largely in the protoplasm or distributed throughout the cell.

The slope of the regression line for protein nitrogen on cell volume was steeper than that for soluble nitrogen and suggested that the proportion of protein increased with cell size increase.

(*d*) *Jonathan Spot*

There was a complex relationship between Jonathan spot and breakdown which has not been reported before. While there was a negative correlation

($P < 0{\cdot}001$) between the two disorders between trees there was a positive association, i.e. more fruit with joint symptoms than would be expected by chance* ($P < 0{\cdot}001$), but the relation between the two disorders will not be discussed as no theory can be advanced to resolve the apparent paradox.

TABLE 3

PARTIAL CORRELATIONS OF VARIABLES

Correlation	Constant	r	Significance
Protein N per cell and cell surface ..	Mean fruit size	0·8260	$P<0{\cdot}001$
Protein N per cell and respiration per cell	Mean fruit size	0·8283	$P<0{\cdot}001$
Protein N per cell and R/P ratio ..	Mean fruit size	0·2066	N.S.
Protein N per cell and soluble N per cell	Mean fruit size	0·7414	$P<0{\cdot}001$
Protein N per cell and percentage breakdown	Mean fruit size	0·3050	N.S.
Percentage disorder and R/P ratio ..	Mean fruit size	0·2655	N.S.
Percentage breakdown and R/P ratio ..	Mean fruit size	0·1468	N.S.
Percentage disorder and mean fruit size ..	Respiration/protein	0·7795	$P<0{\cdot}001$
Percentage breakdown and mean fruit size	Respiration/protein	0·7007	$P<0{\cdot}001$
Percentage breakdown and mean fruit size	Cell volume	0·6120	$P<0{\cdot}001$
Percentage breakdown and cell volume ..	Mean fruit size	0·0943	N.S.
Respiration per cell and protein N per cell	Cell number/g	0·8225	$P<0{\cdot}001$

IV. DISCUSSION

This paper attempts to test for a wide range of crop size within a variety the indications received from the studies of different varieties at two cropping levels (Martin and Lewis 1952) and to discover something of the physiological connection between mean fruit size per tree and susceptibility to disorder (Martin 1954).

Many of the interrelations of cell attributes which were demonstrated in the first-mentioned of these papers for cell characteristics between varieties are now shown to hold for cell size differences within a variety.

* Calculated as follows: Expected percentage for joint occurrence on chance basis $= \frac{\text{Jonathan spot (\%)} \times \text{Breakdown (\%)}}{100}$

Σ individuals^2 (actual$-$expected) $= 564{\cdot}87$

G.T. (actual$-$expected)$^2 30 = 198{\cdot}18$

Source	D.f.	Sum of Squares	Mean Square	F	Significance
G.T.	1	198·15	198·15	15·67	$P<0{\cdot}001$
Error	29	366·72	12·645		

(*a*) *Mean Cell Volume, Cell Surface, and Protein Nitrogen*

The relation between cell size and protein content has now been demonstrated for within-tree development (Robertson and Turner 1951); between varieties (Martin and Lewis 1952); and now for between-tree variations within a variety. The principle that protein synthesis keeps pace with cell enlargement is thus a characteristic of apple cells under a wide range of conditions.

Kidd *et al.* (1951), by other methods, have demonstrated a decrease in protoplasm thickness as apple cells expand. Unless their conditions were exceptional and cell size increase without accompanying protein synthesis occurred, the two results can be reconciled only by assuming that protein nitrogen becomes more concentrated in the protoplasm as the cells expand.

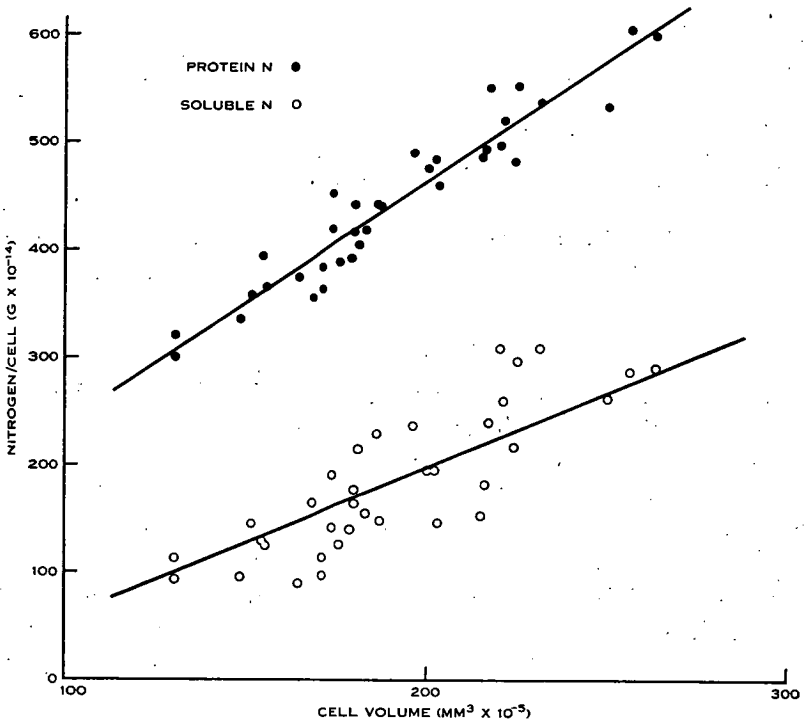

Fig. 2.—Protein and soluble nitrogen and cell volume.

(*b*) *Protein Nitrogen, Soluble Nitrogen, and Respiration*

The close relation between protein nitrogen and respiration is now shown to be a characteristic of cell growth within a variety, as well as between varieties.

Robertson and Turner (1951) suggested a "steady state" relation between protein and soluble nitrogen in their studies of cell enlargement during fruit growth. The very close correlation now shown for these two variables between

trees, which remains highly significant ($P < 0{\cdot}001$) when mean fruit size per tree is held constant, supports their suggestion.

The positive correlation of protein nitrogen per cell with respiration per unit protein would be consistent with another of their suggestions, that increased protein content of cell is associated with increased difficulties of protein maintenance and respiration rate. This may be linked with the possibility of an increase in protein concentration suggested in (*a*) above.

The decline in significance of the relation between respiration per unit protein and protein nitrogen per cell when fruit size per tree is held constant may be due to the difference in level of experimental error of the different terms.

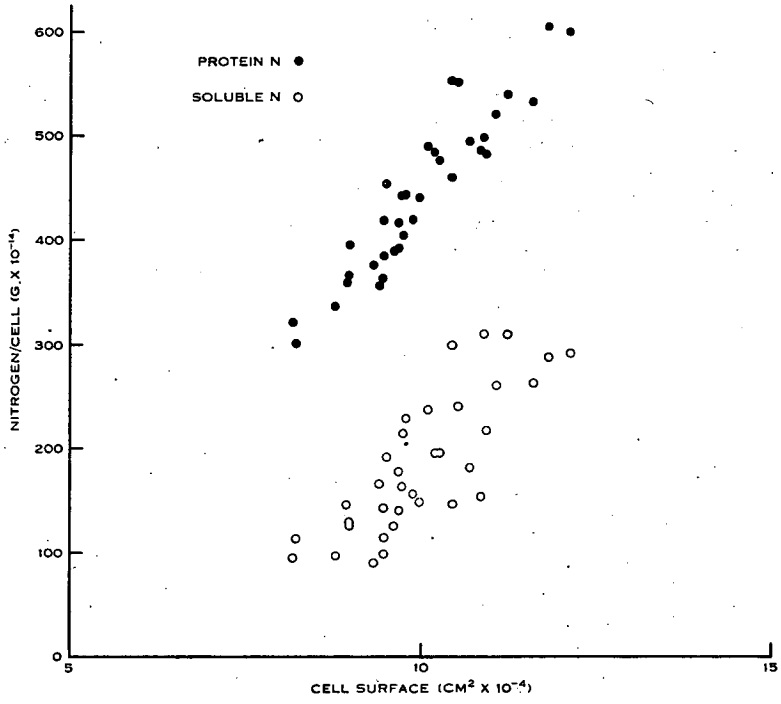

Fig. 3.—Protein and soluble nitrogen and cell surface.

(*e*) *Breakdown, Respiration per Unit Protein, and Cell Size*

The earlier suggestion that variation in disorder level might be related to difficulties of cell organization associated with cell growth has been supported by the intercorrelation of these facts. The decline in significance when mean fruit size is held constant by methods of partial correlation may be due to differences in level of precision in the different terms; the partial correlation of disorder and mean fruit size for constant respiration per unit protein may remain significant for the same reason.

If the differences in level of precision are not responsible for the magnitudes of the partial correlations, the explanation of why mean fruit size is such

a good index of disorder susceptibility does not lie in the level of efficiency of respiration in cells of different sizes. However, the probability that light-crop trees not only have fewer fruit buds but may also have less cell division in the fruits in spite of their larger size, suggests weakness in cell organization that merits further study.

V. Acknowledgments

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LOW OXYGEN STORAGE OF APPLES

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LOW OXYGEN GAS STORAGE TRIALS OF APPLES IN TASMANIA

By D. MARTIN* and J. CERNY*

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Summary

Gas storage mixtures of the conventional type containing 5 per cent. carbon dioxide and 16 per cent. oxygen have proved unsatisfactory for many apple varieties grown in Tasmania because of increased susceptibility to scald and breakdown. The use of higher temperatures to avoid these disorders reduced the value of this method and gave little advantage over air storage at lower temperatures.

Low oxygen concentrations in the absence of carbon dioxide have allowed the use of low storage temperatures without increasing the susceptibility to low-temperature breakdown and have given good control of softening, superior texture, and reduced wastage from disorders and rots, and have markedly reduced the proportion of soluble pectin. Concentrations of oxygen of the order of 3 per cent. appear likely to give the best results. The method proved suitable for the varieties Cox, Cleopatra, Granny Smith, Delicious, Golden Delicious, Tasman Pride, Geeveston Fanny, Jonathan, Democrat, Legana, and Sturmer, and the pear variety Packham's Triumph. It was superior to gas mixtures containing 5 per cent. carbon dioxide and 16 per cent. oxygen for apple varieties susceptible to scald and breakdown and at least as good for varieties resistant to those disorders. Control of Jonathan spot and pit was inferior to carbon dioxide gas storage but superior to air storage.

I. INTRODUCTION

Gas storage of apples in mixtures of carbon dioxide and oxygen in different proportions and at temperatures varying with the variety has become normal commercial practice in England and has been the subject of considerable experiment in many other countries including Australia, and many pamphlets giving practical instruction have been issued, e.g. Phillips (1946) in Canada, Smock (1949) in U.S.A., and Huelin and Tindale (1947) in Australia.

Data collected during earlier studies on brown heart (Martin and Carne 1950) and some further experiments carried out in 1951 (see Table 1) showed that breakdown and scald were increased in 5 per cent. carbon dioxide, 16 per cent. oxygen. This was particularly striking in Tasman Pride variety, where increase in temperature from 33 to 37 °F did not reduce breakdown incidence.

Jonathan variety, which has given satisfactory results in gas storage in Victoria (Huelin and Tindale 1947) and proved satisfactory in 1951 in Tasmania, developed severe breakdown in 1952 even when the temperature change technique of these authors was adopted. Cleopatra variety, in which breakdown is extremely rare, developed the disorder in 1940 and 1951.

Although the effect of carbon dioxide increasing incidence of breakdown has been reported by other workers (Kidd and West 1927, 1930; Tiller 1932; Eaves 1938; Huelin and Tindale 1947), they generally found it possible to avoid this effect by raising the storage temperature.

* Division of Plant Industry, Tasmanian Regional Laboratory, Hobart.

In Tasmania, trials with carbon dioxide gas storage have proved most consistently successful with varieties like Democrat which have such a low susceptibility

TABLE 1

DISORDER INCIDENCE IN CARBON DIOXIDE GAS STORAGE

Variety		CO_2 (%)	O_2 (%)	33 °F		35 °F		37 °F	
				Breakdown (%)	Scald (%)	Breakdown (%)	Scald (%)	Breakdown (%)	Scald (%)
Cleopatra	1940	5	16	3·5	0				
		0	21	0	0				
	1951	5	16	2·1	85·6			15·2	84·3
		0	21	0·0	53·0			0·0	52·0
Delicious	1940	5	16	7·0				10	
		0	21	0				0	
Geeveston Fanny	1939	5	16	35·0				10	
		0	21	5·0				0	
	1941	5	16	12·0				10·0	
		0	21	5·0				5·0	
	1951	5	16	8·5		2·8		2·6	
		0	21	0·9		0·0		0·0	
Granny Smith	1951	5	16	0	43·8			0·0	61·5
		0	21	0	22·4			0·0	22·0
Jonathan	1952	5	16	72·4		50·4		47·5	
		0	21	49·6		32·2		23·8	
		5	16	76·6*					
		0	21	48·3					
Tasman Pride	1938	5	16	34·0				46·0	
		0	21	0·0				0·0	
	1940	5	16	14·0				0·0	
		0	21	0·0				0·0	
	1951	5	16	37·8				33·5	
		0	21	1·7				1·2	
Sturmer	1937	5	16	10	10	0	16	0	38
		0	21	0	2	0	0	0	0
	1941	5	16	35	0			0	0
		0	21	0	0			0	0

* 37 °F for 8 weeks, then 33 °F for 8 weeks.

to breakdown that the presence of carbon dioxide did not induce the disorder at low temperatures. With varieties of high or even intermediate susceptibility, the

presence of carbon dioxide either increased the danger of breakdown and scald to such a degree that the method either could not be recommended for commercial application or required the use of such high storage temperatures that there was no advantage over air storage.

The pioneers of the gas storage method favoured the presence of carbon dioxide in the gas mixture (Kidd and West 1937) because of its effect in retarding softening and colour change and reducing fungal rots. They found that lowering the oxygen concentration had relatively less effect. However, there are many references in the literature which suggest that it might be possible to combine the effects of low oxygen concentration with those of low temperature to retard ripening substantially without harmful effects. Kidd and West (1939) gave figures which showed that the total loss of substrate in 2·5 and 5 per cent. oxygen at 1 °C was not significantly greater than in 5 and 10 per cent. carbon dioxide at 5 °C and they recorded (1927) that the reduction of oxygen concentration to 3 per cent. delayed the onset of breakdown. Eaves (1938) found that no breakdown developed in Cox on 2·5 per cent. oxygen, 0 per cent. carbon dioxide compared with 100 per cent. breakdown in a 5 per cent. carbon dioxide, 16 per cent. oxygen mixture, and in the former mixture they found that fungal activity was retarded and high osmotic pressure, percentage total solids, and moisture content were retained. Trout *et al.* (1942) found that reduced respiration in Granny Smith was correlated with decreased internal oxygen but not with increased internal carbon dioxide, and that final colour was closely related to internal oxygen.

Low oxygen concentrations had also effects on other respiratory products which might be beneficial in storage. Hansen (1942) found that they inhibited the production of ethylene, and Kidd and West (1934) showed that the ripening effect of ethylene diminished with the reduction of the concentration in the storage atmosphere. Potter and Griffiths (1947) found that the production of odorous volatiles was reduced by low oxygen tension, which suggested that the production of the particular volatile that causes scald might also decline.

This background suggested that, as carbon dioxide gas storage had proved to have limited application in Tasmania, trials with low oxygen storage should be made. These have been exploratory trials to test a wide range of varieties in a single mixture compared mainly with air storage, though some comparisons with 5 per cent. carbon dioxide and 16 per cent. oxygen have been included. No equipment was available to extend the range of gas concentrations to cover concentrations containing both low oxygen and low carbon dioxide.

Since this investigation was commenced, van Doren (personal communication 1954) has reported success with Golden Delicious and Red Delicious with low concentrations of oxygen (2 per cent.) and a minimum of carbon dioxide. In atmospheres where carbon dioxide was maintained he found that the occurrence of core breakdown and softening of the flesh was in direct proportion to increasing percentages of carbon dioxide so that at 5 per cent. carbon dioxide some injury occurred in 3 or 4 months. Hall and Sykes (1954) have shown a direct relation between carbon dioxide concentration and the incidence of scald.

II. Methods

Storage was carried out in drums of 44-gallon capacity with clamp-on lids such as are normally used for the storage and transport of powders. These were modified as illustrated in Figure 1.

These drums were held in refrigerated chambers to give internal temperatures of 32, 35, and 37 °F in 1952, and 31, 33, and 35 °F in 1953-54. Temperatures were measured by thermocouples. Fine bore sampling tubes led outside the chamber

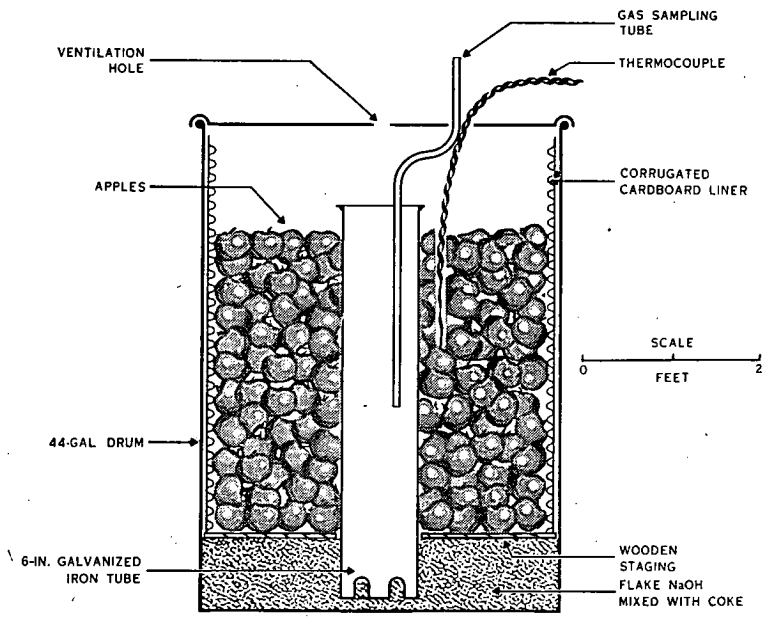

Fig. 1.—Storage drum.

and gas concentrations were determined daily by means of the Orsat-Fisher apparatus, with an accuracy of 0·2 per cent., and adjusted by a ventilation hole in the lid of the drum. The figures given are the mean concentration over the whole period.

Difficulties met in maintaining low oxygen levels appear to be due mainly to the "breathing" of the containers with the cycles of cooling and heating of the refrigeration machinery and with rise and fall of barometric pressure. Leakage of oxygen due to these causes is more than the respiration of the fruit can use at the lowest temperatures and from the 3 per cent. oxygen level this tends to rise to 5-6 per cent.

Fruit was picked from one to three trees of apparently equal growth and mean fruit size, and thoroughly mixed and stored within 48 hr of picking. A sample of

approximately 500 fruits was used, and with samples of this size differences in disorder level of 5 per cent. have proved significant.

After removal from store, the fruit was held for 2 weeks at room temperature before final examination.

Pressure was measured by penetrometer on 20 fruits and colour was estimated by eye on a scale 1-4 from full green to full yellow. Differences in firmness of 0·5 lb are considered significant.

Soluble pectin content was determined by the method of Emmett and Carré (1926).

Tasting tests were carried out by three experienced observers, but not on a statistical basis.

TABLE 2

CLEOPATRA: CONDITION AFTER GAS STORAGE FOR 32 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 14.iii.52

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Scald (%)	Lenticel Spot (%)	Rots (%)	Press. (lb)	Colour
32	2·50	Oil	5·4	0	12·4	26·5	9·8	12·1	3·5
		Plain	5·4	0	33·1	23·1	18·7	11·4	3·5
		Oil	21	0	52·9	1·4	27·1	11·8	3·0
		Plain	21	0	56·0	..	42·4	10·3	3·0
35	2·50	Oil	5·5	0	17·7	1·2	10·6	11·1	3·5
		Plain	5·5	0	41·3	6·0	8·3	10·8	3·5
		Oil	21	0	70·0	0·0	10·0	11·5	3·5
		Plain	21	0	72·7	1·5	27·3	11·2	3·0
37	2·50	Oil	4·7	0	15·2	14·0	8·7	11·7	3·5
		Plain	4·7	0	65·0	6·5	14·6	11·4	3·5
		Oil	21	0	70·0	0·0	10·0	11·5	3·5
		Plain	21	0	72·7	1·5	27·3	11·2	3·0

III. RESULTS

(*a*) *Cleopatra* (Table 2)

Fruit held in low oxygen storage had improved juiciness and general eating quality, but after 2 weeks at room temperature the fruit was slightly yellower than the controls. Scald and rot wastage was reduced, particularly in oil wraps, but lenticel spot was increased, particularly at 32 °F.

(*b*) *Cox* (Table 3)

From low oxygen storage the fruit was much firmer, juicier, and of higher eating quality than that from air storage, but there was no reduction in pit incidence (in 1953), colour change, or rots. The very low incidence of breakdown was unusual for this variety.

TABLE 3

COX: CONDITIONS AFTER GAS STORAGE FOR 10 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Break-down	Pit (%)	Rots (%)	Press. (lb.)	Colour	Soluble Pectin (%)
Picked 27.ii.53										
31	2·25	..	5	0	0·2	33·4	11·5	15	2·5	0·120
			21	0	1·2	26·9	19·3	13	2·5	0·179
33	2·25	..	5	0	..	51·0	10·2	15	2·5	0·128
			21	0	1·1	50·3	7·3	13	2·5	0·163
35	2·25	..	5	0	0	49·8	9·5	15	2·5	0·126
			21	0	1·2	41·0	12·7	12	2·5	0·172
Picked 25.ii.54										
31	2·15	Plain	3·2	0	..	0·2	2·1	18	2·5	0·090
		Oil	3·2	0	..	0·0	1·2	15	2·5	0·095
		Plain	21	0	..	7·7	3·4	16	2·5	0·180
		Oil	21	0	..	7·4	2·1	14	2·5	0·185
33	2·15	Plain	3·4	0	..	2·0	1·0	16	3·0	0·100
		Oil	3·4	0	..	0·6	1·0	14	3·0	0·105
		Plain	21	0	..	5·0	1·5	14	3·0	0·190
		Oil	21	0	..	3·8	1·5	12	3·0	0·190

(*c*) *Crofton* (Table 4)

Shrivelling was severe in air storage and the condition of gas stored fruit was superior in this respect, but there was no significant difference in wastage at 31 and 33 °F. In carbon dioxide gas storage at 35 °F rotting was severe.

(*d*) *Delicious* (Table 5)

With this variety difficulties were met in reducing the oxygen to a satisfactory level, but the treatment gave a marked improvement in texture and quality. Rotting was reduced and there was some retarding of colour change and softening.

(*e*) *Democrat* (Table 6)

Increase in firmness and juiciness in low oxygen storage was marked; there was also a slight reduction in rotting and colour change. These qualities were probably related to the lower soluble pectin content. Carbon dioxide gas storage

TABLE 4

CROFTON: CONDITION AFTER GAS STORAGE FOR 34 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 20.iv.53

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Rots (%)	Press. (lb)	Colour	Soluble Pectin (%)
31	2·25	Plain	5	0	9·5	17·0	2·0	0·033
		Oil	5	0	13·0	16·8	2·0	
		Plain	21	0	5·5	16·5	2·0	0·061
		Oil	21	0	6·0	16·5	2·0	
33	2·25	Plain	5	0	8·4	16·5	2·0	0·035
		Oil	5	0	11·1	17·0	2·0	
		Plain	21	0	8·3	16·5	2·0	0·065
		Oil	21	0	11·2	16·5	2·0	
35	2·25	Plain	16	5	28·2	15·6	2·0	0·030
		Oil	16	5	31·2	15·8	2·0	
		Plain	21	0	3·5	15·7	2·0	0·070
		Oil	21	0	8·7	16·1	2·0	

TABLE 5

DELICIOUS: CONDITION AFTER GAS STORAGE FOR 30 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Break-down (%)	Rots (%)	Press. (lb)	Colour
Picked 2.iv.52								
32	2·50	..	6·8	0	0·0	18·2	13·9	2·0
			21	0	0·5	16·2	12·9	2·5
37	2·50		7·0	0	0·0	18·3	14·0	2·0
			21	0	0·6	30·4	12·1	2·5
Picked 8.iv.54								
32	2·75	Plain	12·0	0	0·0	5·5	14·1	2·0
		Oil	12·0	0	0·0	10·5	14·1	2·0
		Plain	21·0	0	0·0	15·2	13·3	3·0
		Oil	21·0	0	0·0	20·5	13·3	3·0

TABLE 6

DEMOCRAT: CONDITION AFTER GAS STORAGE FOR 34 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 4.v.53

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Rots (%)	Press. (lb)	Colour	Soluble Pectin (%)
31	2·75	Plain	5	0	6·3	18·9	2·0	0·060
		Oil	5	0	5·5	19·7	2·0	
		Plain	21	0	4·7	16·0	3·0	0·216
		Oil	21	0	8·1	15·6	3·0	
33	2·75	Plain	5	0	4·9	19·5	2·5	0·065
		Oil	5	0	7·2	18·9	2·5	
		Plain	21	0	9·5	14·7	3·0	0·220
		Oil	21	0	13·3	15·5	3·0	
35	2·75	Plain	16	5	13·0	16·8	3·0	0·065
		Oil	16	5	11·1	17·1	3·0	
		Plain	21	0	8·0	13·3	3·0	0·223
		Oil	21	0	7·3	13·4	3·0	

TABLE 7

GEEVESTON FANNY: CONDITION AFTER GAS STORAGE FOR 30 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 27.iii.53

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Core Flush (%)	Rots (%)	Press. (lb)	Colour	Soluble Pectin (%)
31	2·50	Plain	5	0	7·0	15·0	12	3·0	0·150
		Oil	5	0	2·3	15·5	11	3·0	
		Plain	21	0	40·0	16·5	10	3·5	0·247
		Oil	21	0	21·0	12·0	10	3·5	
33	2·50	Plain	5	0	7·0	14·0	11	3·5	0·160
		Oil	5	0	12·0	15·0	11	3·5	
		Plain	21	0	39·0	42·0	9	4·0	0·253
		Oil	21	0	24·0	25·0	9	4·0	
35	2·50	Plain	5	0	2·0	12·0	12	3·5	0·175
		Oil	5	0	17·0	16·0	11	3·5	
		Plain	21	0	57·0	17·0	8	4·0	0·255
		Oil	21	0	59·0	24·0	8	4·0	

at 35 °F was inferior to low oxygen storage at 31 and 33 °F in rot and colour change control, but gave similar control of pectin degradation.

TABLE 8

GOLDEN DELICIOUS: CONDITION AFTER GAS STORAGE FOR 30 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 26.iii.52

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Break-down (%)	Rots (%)	Press. (lb)	Colour
32	2·25	Plain	5·4	0	0	17·4	11·2	2·5
		Oil	5·4	0	0	2·5	11·4	2·5
		Plain	21	0	1·9	56·4	9·9	3·0
		Oil	21	0	2·6	18·5	10·1	3·0
	2·50	Plain	5·4	0	0	21·4	..	2·5
		Oil	5·4	0	0	14·1	..	2·5
		Plain	21	0	1·8	52·6	..	3·0
		Oil	21	0	5·4	44·6	..	3·0
35	2·25	Plain	5·4	0	0	7·7	11·1	3·0
		Oil	5·4	0	0	8·8	10·6	3·0
		Plain	21	0	0	52·7	9·2	3·5
		Oil	21	0	1·4	23·3	9·2	3·5
	2·50	Plain	5·4	0	0	20·5	..	3·0
		Oil	5·4	0	0	19·1	..	3·0
		Plain	21	0	0	76·5	..	3·5
		Oil	21	0	0	39·1	..	3·5
37	2·25	Plain	7·3	0	0	26·3	10·7	3·0
		Oil	7·3	0	0	10·0	11·2	3·0
		Plain	21	0	0	31·7	9·9	3·5
		Oil	21	0	0	31·6	9·7	3·5
	2·50	Plain	7·3	0	0	20·5	..	3·0
		Oil	7·3	0	0	19·5	..	3·0
		Plain	21	0	0	49·0	..	3·5
		Oil	21	0	0·7	20·5	..	3·5

(*f*) *Geeveston Fanny* (Table 7)

There was a marked reduction in core flush and pectin degradation and a small reduction in rotting and colour change compared with air storage; firmness, juiciness, and general quality were much higher.

TABLE 9

GRANNY SMITH: CONDITION AFTER GAS STORAGE FOR 32 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Scald (%)	Rots (%)	Press. (lb)	Colour	Soluble Pectin (%)
Picked 30.iv.52									
32	2·50	Plain	6·3	0	63·4	15·4	15·3	2·5	
		Oil	6·3	0	2·2	6·7	15·2	2·0	
		Plain	16	5	100	25·0	15·0	2·5	
		Oil	16	5	27·2	0·0	15·2	2·5	
		Plain	21	0	28·6	14·2	13·7	2·5	
		Oil	21	0	0·0	12·5	12·9	2·5	
	2·75	Plain	6·3	0	76·8	4·1	15·2	2·5	
		Oil	6·3	0	8·0	6·0	15·3	2·0	
		Plain	16	5	91·0	33·8	15·0	2·5	
		Oil	16	5	15·9	32·8	15·1	2·5	
		Plain	21	0	9·4	16·7	12·0	2·5	
		Oil	21	0	0·0	17·9	13·5	2·5	
35	2·50	Plain	7·0	0	7·6	26·4	14·1	2·5	
		Oil	7·0	0	2·6	15·4	13·8	2·5	
		Plain	16	5	57·2	14·2	14·1	2·5	
		Oil	16	5	10·5	37·5	14·9	2·5	
		Plain	21	0	0·0	19·3	12·2	2·5	
		Oil	21	0	0·0	20·0	12·2	2·5	
	2·75	Plain	7·0	0	2·6	26·3	13·5	2·5	
		Oil	7·0	0	2·4	9·5	13·5	2·5	
		Plain	16	5	67·2	14·7	14·0	2·5	
		Oil	16	5	7·8	9·2	14·5	2·5	
		Plain	21	0	9·1	18·2	12·1	2·5	
		Oil	21	0	0	18·2	11·9		
Picked 14.iv.53									
31	2·50	Oil	5	0	0	6·2	18·4	2·0	0·060
		Oil	21	0	0	11·6	17·0	2·0	0·110
33	2·50	Oil	5	0	0	5·7	16·6	2·0	0·070
		Oil	21	0	0	10·8	14·1	2·0	0·115
35	2·50	Oil	16	5	0	3·8	17·7	2·0	0·060
		Oil	21	0	0	20·1	14·1	3·0	0·125
Picked 20.iv.54									
31	2·50	Plain	8·4	0	22·4	1·6	15·6	1·5	0·053
		Oil	8·4	0	7·4	2·2	..	1·5	
		Plain	21	0	23·5	4·4	14·1	1·5–2·0	0·105
		Oil	21	0	1·5	3·8		1·5–2·0	

TABLE 9 (*Continued*)

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Scald (%)	Rots (%)	Press. (lb)	Colour	Soluble Pectin (%)
33	2·50	Plain	5	0	27·6	0·5	13·9	1·5	0·060
		Oil	5	0	0·5	1·0	..	1·5	
		Plain	21	0	52·1	10·9	12·3	2·0	0·109
		Oil	21	0	1·8	5·8	..	2·0	
34	2·50	Plain	16	5	92·8	1·3	18·4	1·5	0·034
		Oil	16	5	18·4	3·9		1·5	
		Plain	21	0	61·1	2·7	12·2	2·0	0·126
		Oil	21	0	17·0	8·2		2·0	

(*g*) *Golden Delicious* (Table 8)

In low oxygen storage softening and colour change were retarded, texture and quality improved, and incidence of rots reduced, particularly in oil wraps. There was a development of breakdown in air storage at 32 °F; this disorder is unusual in this variety.

(*h*) *Granny Smith* (Table 9)

In 1953 no scald developed in any treatment; this was unusual and probably due to spray russetting. In carbon dioxide storage scald was severe and greater than in low oxygen storage or air storage. In 1952 there was more scald in low oxygen than in air, but this was not so in 1954. Oil wraps reduced scald in all types of storage. In low oxygen, firmness, juiciness, and quality were enhanced and colour and pectin change retarded.

(*i*) *Jonathan* (Table 10)

Low oxygen storage was superior to carbon dioxide storage in control of breakdown, deep scald, firmness, and juiciness but inferior in the control of Jonathan spot, although superior to air storage.

Results of 1955 treatments on deep scald of Jonathan are given in Appendix I.

(*j*) *Legana* (Table 11)

This very good keeping variety showed no great differences in disorder development in the different types of storage. The most significant difference was in the reduction in pectin degradation and maintenance of crispness and juiciness, which was as high in low oxygen storage as in carbon dioxide storage.

(*k*) *Sturmer* (Table 12)

This variety is so sensitive to carbon dioxide that no experiments using this gas were thought necessary. Low oxygen storage produced a marked improvement in texture and quality, rotting was reduced and firmness maintained at the higher temperatures but there was no other marked effect.

(*l*) *Tasman Pride* (Table 13)

The fruit was over-stored in this experiment but there remained enough difference to show that low oxygen storage was superior to air storage in reducing

TABLE 10

JONATHAN: CONDITION AFTER GAS STORAGE FOR 32 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Break-down (%)	Deep Scald (%)	Jonathan Spot (%)	Rots (%)	Press. (lb)	Colour
Picked 16.iii.53										
31	2·25	..	5	0	1·7	0	76·7*	10·3	12·1	2·0
		..	16	5	17·2	0	0	15·7	10·0	2·0
		..	21	0	1·2	6·2	92·0	10·6	10·3	4·0
33	2·25	..	5	0	0·7	0	36·9*	7·2	11·3	3·5
		..	16	5	14·1	0	0	8·5	9·5	3·5
		..	21	0	0·5	0·6	79·3	11·7	10·2	4·0
35	2·25	..	5	0	2·0	0	25·1*	11·0	10·7	3·5
		..	16	5	9·3	0	0	9·6	10·1	3·5
		..	21	0	0·6	0	69·6	8·6	9·4	4·0
Picked 23.iii.54										
31	2·25	Plain	6·1	0	0	0	84·0	0	10·4	3·0
		Oil	6·1	0	0	0	88·5	0	10·3	3·0
		Plain	21	0	0	2·0	100	2·0	10·0	3·0
		Oil	21	0	0	0	88·5	2·0	9·8	3·0
32	2·75	Plain	12·5	0	2·8	46·0	41·5	5·1	..	3·0
		Oil	21	0	1·1	1·8	50·5	5·3	..	3·0
33	2·25	Plain	4·9	0	0	0	73·0	0·8	10·6	3·0
		Oil	4·9	0	0	0	54·3	0·8	10·8	3·0
		Plain	21	0	0	0	73·4	5·0	10·4	3·5
		Oil	21	0	0	0	76·5	3·8	10·3	3·5
34	2·25	Plain	4·1	0	0	0	49·5	2·4	10·3	3·0
		Oil	4·1	0	0	0	36·0	2·4	10·3	3·0
		Plain	16	5	1·0	0	..	..	10·1	3·0
		Oil	16	5	0	0	...	..	9·6	3·0
		Plain	21	0	0	0	83·0	2·0	10·3	3·5
		Oil	21	0	0	0	87·0	1·0	10·3	3·5

* Very slight.

core flush, flesh browning, softening, and colour change. The lower wastage at 33 and 35 °F compared with 31 °F, was confined to this variety, indicating an exceptional sensitivity. At the higher temperatures rots were increased by oil wraps.

TABLE 11

LEGANA: CONDITION AFTER GAS STORAGE FOR 30 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 5.v.54

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Deep Scald (%)	Rots (%)	Press. (lb)	Colour	Soluble Pectin (%)
31	2·5	Plain	6·3	0	0	0	14·6	1·5	0·032
		Oil	6·3	0	0	0	13·7	1·5	
		Plain	21	0	0	0	14·8	1·5–2·0	0·057
		Oil	21	0	1·3	0	13·5	1·5–2·0	
33	2·5	Plain	3·7	0	0·5	1·1	15·0	1·5	0·024
		Oil	3·7	0	0·4	0·4	15·2	1·5	
		Plain	21	0	3·1	2·5	12·7	1·5–2·0	0·054
		Oil	21	0	0·5	0	12·4	1·5–2·0	
34	2·5	Plain	16	5	0	1·6	13·0	1·5	0·031
		Oil	16	5	0	2·0	13·2	1·5	
		Plain	21	0	0	1·3	13·1	2·0	0·077
		Oil	21	0	0	0·7	13·1	2·0	

TABLE 12

STURMER: CONDITION AFTER GAS STORAGE FOR 30 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 5.v.52

Temp. (°F)	Size (in.)	O_2 (%)	CO_2 (%)	Scald (%)	Rots (%)	Press. (lb)	Colour
35	2·50	8	0	1·4	13·6	12·9	3·5
		21	0	1·4	13·6	12·9	3·5
37	2·50	7	0	0·5	5·5	15·9	3·5
		21	0	1·5	27·7	12·1	3·5

TABLE 13

TASMAN PRIDE: CONDITION AFTER GAS STORAGE FOR 25 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 2.iv.53

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Core Flush (%)	Flesh Browning (%)	Rots (%)	Press. (lb)	Colour
31	2·50	Plain	5	0	80	70	39	12	3·5
		Oil	5	0	80	70	42	13	3·5
		Plain	21	0	100	100	42	9	4·0
		Oil	21	0	100	100	48	9	4·0
33	2·50	Plain	5	0	25	20	26	15	3·0
		Oil	5	0	23	21	36	15	3·0
		Plain	21	0	80	31	43	14	3·5
		Oil	21	0	75	32	46	14	3·5
35	2·50	Plain	5	0	14	8	27	16	3·0
		Oil	5	0	0	0	56	15	3·0
		Plain	21	0	65	39	42	13	3·5
		Oil	21	0	59	32	69	14	3·5

TABLE 14

PACKHAM'S TRIUMPH (PEAR): CONDITION AFTER GAS STORAGE FOR 22 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 25.iii.54

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Scald (%)	Break-down (%)	Brown Core (%)	Rots (%)	Colour
31	2·50	Plain	6·5	0	0	0	0	6·1	1·5
		Oil	6·5	0	0	0	0	1·6	1·5
		Plain	21	0	11·5	0	0	14·5	2·0
		Oil	21	0	0	0	0	8·0	2·0
33	2·50	Plain	3·7	0	6·1	0	0	10·2	2·0
		Oil	3·7	0	6·1	0	0	9·8	2·0
		Plain	21	0	20·4	26·0	5·6	35·2	2·0
		Oil	21	0	11·6	24·6	23·2	18·9	2·0
34	2·50	Plain	16	5	0	0	0	5·0	2·0
		Oil	16	5	0	0	0	0	2·0
		Plain	21	0	86·4	26·0	8·6	58·0	3·0
		Oil	21	0	10·0	23·7	30·5	37·5	3·0

(*m*) *Packham's Triumph* (*Pear*) (Table 14)

Low oxygen storage was superior to air storage in controlling ripening and the pear disorders associated with it: scald, breakdown, brown core, and rots. It was, however, no better than carbon dioxide storage in preventing disorders and retarding ripening at 31 °F, and was inferior at higher temperatures. The fruit ripened normally to excellent quality without the astringency associated with the skin that is common with pears in long storage. There was a reduction in scald and rots in oil wraps indicating some further retarding of ripening.

IV. Conclusions

For Tasmanian fruit, storage in low concentrations of oxygen and in the absence of carbon dioxide at 31-32 °F was much superior to air storage in maintaining texture and quality and controlling the incidence of physiological storage disorders. It was as satisfactory as the 16 per cent. oxygen, 5 per cent. carbon dioxide mixture at 35 °F and higher temperatures in extending storage life and gave better control of breakdown, scald, and core flush, but was inferior in the control of Jonathan spot and probably pit.

The effect on colour change varied with the variety in Cleopatra, Cox, Granny Smith, and Golden Delicious. There was little retarding compared with air storage, in spite of large effects on other ripening changes, such as firmness and soluble pectin production; in other varieties colour change was retarded but not substantially.

The figures for firmness and soluble pectin content supported the findings of Eaves (1938), that low oxygen storage reduced the rate of pectin degradation. This reduction was similar to that obtained in carbon dioxide storage.

No "off" flavours developed in low oxygen storage; the level of flavour characteristic of the variety was low immediately on removal, and a week in air was sufficient for this to develop. It is presumed that this was due to the lowered production of volatiles in low oxygen concentrations as reported by Potter and Griffiths (1947).

The pear variety Packham's Triumph kept better than in air (but no better than in carbon dioxide storage), and ripened normally to excellent flavour and condition.

The method appears to have useful features for commercial application. It permits the use of low storage temperatures while avoiding the dangers of those physiological disorders associated with carbon dioxide. A single level suitable for all varieties appears possible, and 3 per cent. oxygen at 32 °F is suggested. The complete removal of carbon dioxide and controlling oxygen level alone should be simpler than controlling both oxygen and carbon dioxide in scrubbed gas stores.

The mechanical difficulties of maintaining low oxygen concentrations are considerable but studies in this field are in hand (Pflug and Southwick 1954).

A variation has been noted in the difference in rot development in oil compared with oil wraps; on some occasions rotting has been greater in oil wraps, on other occasions it has been less.

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Appendix I

The Effect of Treatment on Deep Scald of Jonathan

Results from 1955 treatments (Table 15) have become available earlier than expected and the incidence of deep scald has been exceptionally high; sufficiently high to give significant effects of oil wrap and low carbon dioxide treatments.

Incidence of deep scald decreased with increasing temperatures over the range 30-40 °F, confirming for this range the experience of many earlier workers for somewhat higher ranges. Significant reduction occurred with the use of oil wraps and low oxygen treatment. Incidence was less in low oxygen treatment than in carbon dioxide treatment. These results suggest that the disorder may be of the nature of a volatile injury.

TABLE 15

JONATHAN: CONDITION AFTER GAS STORAGE FOR 16 WEEKS, FOLLOWED BY 2 WEEKS IN AIR AT ROOM TEMPERATURE

Picked 17.iii.55

Temp. (°F)	Size (in.)	Wrap	O_2 (%)	CO_2 (%)	Deep Scald (%)	Press. (lb)	Colour
30	2·37	Plain	21	0	91·5	13·3	2·5
		Oil	21	0	92·9	11·8	2·5
		Plain	5	0	22·2	13·5	2·5
		Oil	5	0	14·4	12·2	2·5
32	2·37	Plain	21	0	76·2	12·0	2·5
		Oil	21	0	60·0	10·6	2·5
		Plain	5	0	14·7	12·9	2·5
		Oil	5	0	4·1	11·8	2·5
34	2·37	Plain	21	0	77·0	11·2	3
		Oil	21	0	64·8	11·0	3
		Plain	16	5	21·1	11·6	3
		Oil	16	5	3·3	12·0	3
		Plain	5	0	0·5	11·3	3
		Oil	5	0	0·0	11·8	3

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BITTER PIT IN THE APPLE VARIETY CLEOPATRA IN TASMANIA IN RELATION TO CALCIUM AND MAGNESIUM

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BITTER PIT IN THE APPLE VARIETY CLEOPATRA IN TASMANIA IN RELATION TO CALCIUM AND MAGNESIUM

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Summary

When spray treatments were applied to half-trees of Cleopatra apples, it was shown that magnesium nitrate increased the incidence of pit and calcium nitrate decreased it. There was a suggestion that borax decreased the effectiveness of the calcium nitrate treatment.

Magnesium or calcium nitrate, with or without borax, did not affect the potassium, magnesium, phosphorus, or nitrogen content of the fruit cortex. Calcium nitrate in 1959 increased the calcium content but magnesium nitrate had no effect.

There was no significant difference in the content of potassium, magnesium, or phosphorus between 1958 and 1959, but the calcium content was 3·3 times as high in 1958. Pit incidence was low in 1958 and high in 1959.

No significant difference in content of these four elements could be demonstrated between sound and pitted fruits.

The results support the view that calcium is the critical element in pit incidence and that magnesium may play an important part.

I. INTRODUCTION

The influence of moisture stress, fruit size, crop size, and nitrogen level in determining the incidence of pit in apples is now generally accepted, but the evidence for the effect of mineral elements other than nitrogen is still so conflicting that interest in the possibility that mineral treatment could affect it has declined greatly. Pit is such an important disorder that most apple research centres have experimented with some form of mineral treatment.

Following the discovery of boron deficiency by Atkinson (1935) and McLarty (1936) the superficial resemblance of some of its forms to the lesions of pit led many investigators to test the effect of boron treatments on the latter. The results were variable. Ekstrand (1941), Levy (1947), Mulder (1948), Wiebosch (1948), and van Stiuvenberg and Pouwer (1950) obtained a response in some cases; while McLarty (1936), Atkinson (1937), Burrell (1937), Smock (1937, 1941), Chittenden and Thompson (1938), Cole (1938), Magness (1938), Wallace and Jones (1940), Maier (1941), Martin and Carne (1950), and Mulder (1951) obtained little or no response in others. More recently Dunlap and Thompson (1959) found that sprays of boron applied at full bloom gave a response, but there is doubt that the disorder in this case was similar to that recognized as bitter pit in Australia.

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Of the other elements, Wallace and Jones (1940) and van Stiuvenberg and Pouwer (1950) suggested that excess potassium increased the disorder, and van Shreven (1958) found a significant correlation between fruit potassium and pit incidence after the crop factor had been eliminated by partial correlation; Rose *et al.* (1933) induced symptoms similar to pit with magnesium treatment, and Mulder (1951) thought pit might be connected with low phosphorus due to low magnesium intake; and Delong (1937) found that pitted fruits were lower in calcium than sound fruits. Brown (1926) found that pitted fruits were high in ash content, with a low percentage of phosphorus in the ash.

More recently Garman and Mathis (1956) have carried out experiments on Baldwin spot (a disorder probably identical with pit) which indicated that calcium level was an important factor in determining incidence, and that antagonistic effects could develop between calcium and magnesium or between calcium and magnesium plus potassium. They obtained responses from late summer sprays and from soil injections with solutions at an earlier (unspecified) date. They also advanced the hypothesis that in times of water stress the leaves might compete successfully with the fruit for calcium. Further evidence on the importance of calcium in fruits under conditions of water stress has been given by Geraldson (1957) and Maynard *et al.* (1957) for the disease, blossom end rot of tomatoes. Blackheart of celery has also been shown (Geraldson 1953) to be due to localized calcium deficiency within the plant. The condition could be reduced by solutions of calcium or increased by magnesium salts.

In Tasmania the important commercial varieties Cleopatra and Cox are highly susceptible to pit in certain years, but most study has so far been directed to crop and growth factors (Martin 1953, 1954*a*, 1954*b*).

Some experiments with calcium plus boron had been attempted in earlier experiments. A slight decrease in pit following injections and spray treatment with boron remained when the effects of crop and fruit size had been eliminated (Martin and Carne 1950); but spring and early summer sprays and injections of calcium phosphate had failed to produce any significant responses, and a wide range of soil dressings of lime with and without phosphate had sometimes given increased pit, probably owing to increased growth. Soil treatments with calcium sulphate and calcium chloride had also given no reduction. This failure to get response to dry soil treatments or to early sprays of calcium was the general experience of bitter pit investigators. The suggestion that leaves could compete successfully for calcium at times of water stress made it desirable to re-examine the position. Experiments with calcium, potassium, and magnesium salts as summer sprays were therefore undertaken in 1958 and 1959.

II. Material and Methods

A block of Cleopatra trees (plot 1) with a history of high susceptibility to pit and a stable and balanced manurial programme of 3 cwt per acre per year (N : P : K ratio 2 : 2 : 1) was selected.

Six different spray treatments, viz:

Potassium nitrate,
Magnesium nitrate,
Calcium nitrate,
Calcium dihydrogen phosphate,
Calcium dihydrogen phosphate + borax,
Calcium nitrate + borax,

were applied as N/10 solutions at approximately weekly intervals (four applications) from the first week in January 1958 to half-trees; the aspect of the treated half

TABLE 1

FRUIT WEIGHT AND PERCENTAGE OF PITTED FRUIT FOLLOWING TREATMENT

Treatment	Plot 1, 1958		Plot 1, 1959		Plot 2, 1959	
	Mean Fruit Wt. (g)	Pit (%)	Mean Fruit Wt. (g)	Pit (%)	Mean Fruit Wt. (g)	Pit (%)
KNO_3	77·5	0·5	121·2	36·7	106·3	22·8
Control	79·4	0·3	123·6	39·5	108·8	16·1
$Mg(NO_3)_2$	84·2	19·0	119·2	54·1	103·2	12·5
Control	81·8	3·6	119·8	42·7	104·8	4·8
$Ca(NO_3)_2$	85·8	0·6	118·3	6·6	109·4	3·6
Control	85·2	1·1	118·1	35·1	109·1	12·6
$Ca(NO_3)_2$ + borax	78·0	0·6	116·2	23·4	104·6	2·2
Control	79·1	1·3	113·8	41·9	106·9	8·8
$Ca(H_2PO_4)_2$	80·2	0·4	120·7	46·2	107·1	9·5
Control	79·1	1·1	121·1	60·7	106·4	8·8
$Ca(H_2PO_4)_2$ + borax	77·9	1·8	119·8	46·2	107·1	15·1
Control	77·1	1·2	120·6	47·4	113·8	19·0
Nil	81·4	1·7	125·0	49·3		
Control	81·1	1·9	124·4	48·3		

being randomized. The unsprayed half of each tree was used for a control, earlier experience having shown that there was no migration of mineral elements from sprayed to unsprayed halves, or even between major branches of the vase-shaped trees which are usual in Tasmania. There were six replicates of each treatment. Sufficient spray was applied to wet the leaves without substantial drip.

In 1959 the treatments were repeated on the same trees, and the experience was duplicated in another orchard (plot 2).

On March 14 a random sample of approximately 50 lb (150–200 fruits) fruit was picked from the treated and untreated side of each tree and kept separate. After storage at 33–34°F for 6 months, followed by 2 weeks at 65°F, the samples were examined for pit.

Chemical analysis of the dried flesh (excluding peel and core) of representative samples of 30 fruits of the sound and pitted fruits of the treated and untreated sides of the magnesium nitrate trees of plot 1 were made in 1958 and 1959. In 1959, analyses of the fruit of the calcium nitrate and calcium nitrate+borax plots were also made. Potassium, calcium, magnesium, and phosphorus determinations were made by the Analytical Section of the Division of Plant Industry, and nitrogen determinations by the authors.

Potassium and calcium were determined by the method of Williams and Twine (1960), magnesium by the method of David (1958), and phosphorus by the method of Cavell (1955), all following a nitric-perchloric-sulphuric acid digestion for the destruction of organic matter.

III. Results

(*a*) *Pit*

The 1958 season was dry, crops were heavy, and the incidence of pit was very low. In 1959, crops were light and the incidence was quite high. The mean fruit weight and percentage pitted fruit for each treatment and its controls are given in Table 1.

The data for plot 1, 1958 and 1959, and for plot 2, 1959, were analysed separately, differences in angular transformation for treated and untreated half-trees being used. Because there may have been some correlation in performance between 1958 and 1959 within trees due to continuity of factors other than treatment, the differences of the two halves were combined for the two years and analysed. The variances of these totals were in fact almost equal to the sum of the separate variances, which indicated complete or almost complete independence between the two years. The plot results were subsequently combined. The analyses using angular transformations are given in Table 2.

The main results are:

(1) Magnesium nitrate: increased pit ($P<0{\cdot}001$).

(2) Calcium nitrate: decreased pit ($P<0{\cdot}001$).

(3) Calcium dihydrogen phosphate: no significant effect with or without borax.

(4) The difference between calcium nitrate alone and with borax for plots 1 and 2 combined was $3{\cdot}82$, with a standard error of $2{\cdot}34$, so that $t = 1{\cdot}63$, $P = 1/9$. The evidence that the addition of borax reduces the effectiveness of calcium nitrate is therefore not at all conclusive.

(*b*) *Mean Fruit Weight*

The data on differences were analysed, but there was no effect of treatment on mean fruit weight with or without pooling.

(c) *Mineral Content*

The mean content of potassium, calcium,* magnesium, phosphorus, and nitrogen of treated and control sides are given in Table 3. Conversion to percentage fresh weight did not affect any of the comparisons of Table 3 except magnesium, where the difference between content declined from significance at $P<0\cdot05$ on a dry weight basis to insignificance on a fresh weight basis.

There were no significant differences between sound and pitted fruits.

Treatment with magnesium nitrate, calcium nitrate, or calcium nitrate+borax had no significant effect on the content of potassium, magnesium, or phosphorus. Treatment with magnesium nitrate had no significant effect on the level of calcium.

TABLE 2

DIFFERENCES IN PERCENTAGE PIT (ANGULAR TRANSFORMATION)

Control – treated half-trees

Treatment	Plot 1, 1958	Plot 1, 1959	Plot 2, 1959	Composite
	Mean Difference	Mean Difference	Mean Difference	Mean Difference
KNO_3	− 1·50	1·52	− 4·74	− 1·57
$Mg(NO_3)_2$	−15·41***	− 7·17*	− 8·47*	−10·35***
$Ca(NO_3)_2$	2·83	22·48***	10·24**	11·85***
$Ca(NO_3)_2$+borax	2·71	12·24***	9·14*	8·03***
$Ca(H_2PO_4)_2$	3·34	3·16	− 0·89	1·87
$Ca(H_2PO_4)_2$+borax	− 0·18	0·80	3·07	1·23
Nil	0·87	− 0·60	0·27	—

* $P<0\cdot05$. ** $P<0\cdot01$. *** $P<0\cdot001$.

Treatment with calcium nitrate with and without borax in 1959 significantly increased the calcium content of the pulp above that of the controls, but this was still very much below that of even the pitted fruit in the previous year. There was no effect on potassium, magnesium, or phosphorus content.

In comparisons between 1958 and 1959, the most striking feature is the higher calcium content in 1958: this was more than three times as high on a dry weight basis, three times as high on a fresh weight basis, and twice as high on a per fruit basis.

IV. DISCUSSION

When applied as summer sprays, calcium nitrate decreased and magnesium nitrate increased very significantly the incidence of pit in these Cleopatra apples in Tasmania. These results are in keeping with the conclusions of Garman and Mathis (1956) for Baldwin spot in the United States, that: "the critical element

* In 1959 the calcium level was extremely low, in many cases too low for the method used to detect; these cases were treated as zero values.

is calcium and the unbalanced condition lies between calcium and magnesium". Unlike the results for Baldwin spot, there is no evidence from our results that potassium or phosphorus is involved, for potassium nitrate and calcium dihydrogen phosphate did not affect incidence. It is possible that borax applied with calcium nitrate reduced its effectiveness.

While there is some evidence that calcium nitrate sprays affect the calcium level of fruit in years of very low level of pit, magnesium nitrate sprays do not

TABLE 3

MEAN MINERAL CONTENT OF RANDOM SAMPLES OF 30 FRUITS FROM TREATED AND UNTREATED HALF-TREES

Values given as percentage of dry weight

Treatment	Potassium (%)		Calcium (%)		Magnesium (%)		Phosphorus (%)		Nitrogen (%)	
	1958	1959	1958	1959	1958	1959	1958	1959	1958	1959
$Mg(NO_3)_2$	0·88	0·84	0·015	0·0045	0·041	0·033	0·045	0·047	0·29	0·23
Control	0·87	0·86	0·015	0·0045	0·041	0·037	0·045	0·047	0·29	0·24
$Ca(NO_3)_2$		0·84		0·0084		0·039		0·048		0·21
Control		0·86		0·0039		0·033		0·045		0·24
$Ca(NO_3)_2$ + borax		0·89		0·0084		0·037		0·052		0·22
Control		0·82		0·0045		0·035		0·048		0·23
Sound	0·87		0·16		0·40		0·44		0·29	
Pitted	0·88		0·13		0·43		0·47		0·30	

appear to do so and therefore do not operate by depressing the calcium level of the whole fruit. Neither spray affects the level of potassium, phosphorus, or nitrogen.

No explanation can be offered as to why calcium as the nitrate should reduce pit and calcium as the dihydrogen phosphate should not. In view of the fact that nitrogen applications usually increase pit and phosphate has been reported to reduce it, the opposite result might have been expected.

Very wide (up to threefold on a fresh weight basis) fluctuations in the calcium content of fruit can apparently occur between seasons, and this is in marked contrast to the behaviour of potassium, phosphorus, magnesium, and nitrogen under a uniform manurial programme. Such fluctuations do not appear to have been recorded in either leaves or fruit of other varieties, though Ljones (1954) has shown that the potassium content is higher and the calcium and magnesium content lower in light than in heavy crops. Garman and Mathis (1956) also reported that the calcium and magnesium content were lower in light crops and the ratio of these elements could vary between seasons. These differences were, however, not more than twofold.

All that can be said is that application of calcium nitrate solutions reduces those climatic and crop effects which lead to pit lesions, and magnesium nitrate solutions increase them, but there is no real evidence that the magnesium metabolism of the fruit is involved. The probability that calcium metabolism is affected directly rests on the basis of a lowered calcium content in the year of high susceptibility, and a probable increase in calcium content and pit reduction following calcium nitrate sprays. It remains to be discovered whether, in times of water stress, leaves withdraw calcium from the fruit to injuriously low levels, or whether at marginal calcium levels water stress alone results in injury.

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Addendum

Since this article was submitted for publication, Askew *et al.* (1960) have reported, in the Annual Report of the Cawthron Institute, a reduction in bitter pit in Cox's Orange Pippin apples in one New Zealand orchard following sprays of calcium acetate.

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JONATHAN SPOT—THREE FACTORS RELATED TO INCIDENCE: FRUIT SIZE, BREAKDOWN, AND SEED NUMBERS

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By D. MARTIN,* T. L. LEWIS,* and J. CERNY*

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Summary

Observations are reported which show that:

(1) There is an interaction between the disorders Jonathan spot and breakdown. There is a negative correlation between them, but the same fruits tend to be susceptible to both disorders.

(2) In the absence of other disorders there is a positive intercorrelation between percentage Jonathan spot, mean fruit size, and mean seed number both within and between trees.

(3) In the one fruit size group on a tree, fruits with Jonathan spot have a higher mean seed number per fruit than sound fruit, and the seeds have a greater tendency to germinate.

(4) Within trees, a thinning treatment which produces fruit of differing sizes but with the same seed number does not alter the percentage Jonathan spot. Between trees, a thinning treatment which produces fruit of the same size but a differing seed number results in differing levels of Jonathan spot.

I. INTRODUCTION

Wherever the apple variety Jonathan is grown, the disorder Jonathan spot has been recorded. Although similar symptoms affect the varieties Rome Beauty, King David, Worcester, Spitzenberg, Bowden's Seedling, Frimley Beauty, Stayman Winesap, Dougherty, Yellow Newtown, Grimes Golden, and Scarlet, their susceptibility or their commercial importance in Australia is lower. In Tasmania, the disorder often appears in serious amounts after long storage.

Since Brooks and his coworkers (Brooks and Cooley 1917) and later, Plagge and his coworkers (Plagge and Maney 1924; Plagge *et al.* 1935) described the disorder and defined the main factors affecting its incidence, advances have been small. The United States findings have been confirmed by Carne *et al.* (1930), and Carne (1948).

Nothing is known of the fundamental cause of the lesions, but considerable experience has accumulated from the observations of experimenters. In general terms, any conditions which advance maturity favour increased incidence or early appearance of symptoms, while treatments which retard ripening, such as heavy nitrogen manuring, controlled atmosphere storage, or skin coatings (Tindale 1944), reduce it. All workers agree that late picking, delay in cool storage, a slow rate of cooling, and long storage increase it.

There is divergence of opinion on other factors. Brooks and Cooley (1917) considered that storage conditions giving excessive evaporation might induce it, but the experiments of Plagge and Maney (1924) suggested that humidity level had no

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effect, and no consistent differences could be obtained by different levels of aeration. Tomana (1959) demonstrated a negative relationship between incidence and water loss.

Little attention has been directed to the factors of fruit size and crop size. Brooks, Cooley, and Fisher (1920) stated that large apples were more susceptible than small. Plagge and Maney (1924) had inconclusive results; and later workers have generally considered that size was not an important factor but that smaller fruits on heavy crops might be more susceptible. An association of high incidence and a high level of pigmentation has been referred to by the above workers; and as heavy crop fruit has generally more pigment than light, the association might be with the level of pigmentation rather than with the size.

Van Shreven (1958) noted a reduction in incidence following treatment with diphenylamine, but this was not the case in New Zealand (Padfield 1959) or Tasmania (authors, unpublished data).

Recently Poapst and Phillips (1958) have found a connection between seed physiology and the storage disorder core flush. Seeds from fruit with this disorder germinated with greater readiness than seeds from sound fruit, and fruit inoculated with extracts from stratified seeds had an increased susceptibility. No reference to a relationship between seeds and Jonathan spot or between it and other disorders has been noted.

In plots laid down for the study of fruit development in relation to its physiology, Jonathan spot has occurred as a disorder of major importance, usually alone but occasionally in combination with breakdown. Seed counts were begun in 1956 and expanded in 1957 and 1958, and these have revealed unexpected relationships with the disorder which are here reported to draw attention to this factor, which has been neglected in interpreting results of fruit storage experiments.

II. Material and Methods

A block of 150 Jonathan trees on seedling stock, and unusually uniform with respect to tree size, soil type, aspect, and pollinator variety, has been available since 1952, when they were 30 years old. Of these, some have been used for other purposes but the remainder, which included 36 untreated trees, provided material for observations on general relationships affecting disorders under normal conditions of cropping and for certain hand-thinning treatments, viz.:

(1) *Within trees*

Alternate branches thinned heavily on December 20 after most of the natural abscission had occurred (three trees in 1958 only).

(2) *Between trees*

(*a*) Blossom-thinned: clusters thinned to one open blossom* per inflorescence at full bloom (15.x.57) (four trees in 1958 season).

* These flowers would almost certainly have been already pollinated. Each thinned tree was surrounded by unthinned trees, and counts of bees per tree on the thinned and adjacent unthinned trees were made on three occasions during the succeeding week without any difference being found.

(*b*) November-thinned: clusters thinned to one fruitlet per inflorescence on November 21 after the completion of cell division in the fruit (four trees in 1958).

The data collected were based on random samples of 150–200 fruits per tree, which were weighed and counted to find mean fruit weight and stored for 6 months at 33–35°F, followed by 2 weeks at 65°F after which they were examined for storage disorders. Mean seed number per tree was based on a separate random sample of 30 fruits picked at the same time. For within-tree data, the fruits of each tree were divided into four $\frac{1}{4}$-in. size groups corresponding to mean weights of 60, 86, 112, and 138 g; disorder and seed number were examined for each group in 1957 and 1958, and in 1958 the seed numbers were obtained for 50 sound and 50 Jonathan-spotted fruits of two size classes from each tree.

TABLE 1

MEAN FRUIT WEIGHT, MEAN SEED NUMBER PER FRUIT, MEAN PERCENTAGES BREAKDOWN AND JONATHAN SPOT, AND MEAN CROP WEIGHT: 1952–1958

	1952	1953	1954	1955	1956	1957	1958
Mean fruit wt. per tree (g)	98·5	95·4	79·3	76·1	98·4	85·3	77·0
Mean % Jonathan spot per tree	27·3	3·5	0·2	2·1	2·9	53·0	55·0
Mean % breakdown per tree	37·9	3·0	0·3	9·5	1·6	0·3	0·7
Mean seed number per fruit	—	—	—	—	4·11	4·67	5·86
Mean crop wt. per tree (kg)	66·0	63·5	57·0	48·0	96·0	66·5	79·0

III. RESULTS

(*a*) *Interaction of Jonathan Spot and Breakdown*

The general picture of the occurrence of Jonathan spot and breakdown in this plot is given in Table 1, which shows the mean fruit weight per tree, percentage Jonathan spot, percentage breakdown, mean seed number per fruit, and mean crop weight for 1952–1958.

1952 and 1955 were the only years in which breakdown occurred in significant amounts. In 1955 the percentage was moderate only and that of Jonathan spot low, which greatly affected the precision of the relationships in that year. In these years the interaction of the two disorders was examined (see Table 2).

In 1952—when both disorders occurred in reasonable amounts—and also in 1955, the correlation between mean fruit weight and Jonathan spot was positive but not significant; but that between mean fruit weight and breakdown was highly significant; and that between mean fruit weight and the percentage total waste from physiological disorder (fruit with both or either disorder) was slightly greater again.

There was a negative correlation between Jonathan spot and breakdown which was significant in 1952 and remained significant when the mean fruit weight was held constant.

TABLE 2

INTERACTION OF JONATHAN SPOT AND BREAKDOWN: 1952 AND 1955

Values represent mean plus or minus standard deviation

Year	Fruit Wt.	Incidence (%) of Fruit with:			
		Jonathan Spot	Breakdown	Both Disorders	Either or Both Disorders
1952	98·5±18·71	27·3±11·84	37·9±19·31	12·3±0·78	52·9±16·91
1955	76·1±11·14	2·1± 3·99	9·5±16·25	0·09±0·34	11·4±16·69

Correlation	1952		1955	
	r	Signif.	*r*	Signif.
Mean fruit weight and % Jonathan spot	−0·0210	N.S.	+0·0168	N.S.
Mean fruit weight and % Jonathan spot : % breakdown	−0·1124	N.S.	+0·0368	N.S.
Mean fruit weight and % breakdown	+0·6057	$P<0·01$	+0·6841	$P<0·01$
Mean fruit weight and % breakdown : % Jonathan spot	+0·6082	$P<0·01$	+0·6870	$P<0·01$
% Jonathan spot and % breakdown	−0·4122	$P<0·05$	−0·0623	N.S.
% Jonathan spot and % breakdown : mean fruit wt.	−0·4147	$P<0·05$	−0·0769	N.S.

When the actual percentage of fruit with both disorders was compared with the percentage which would be expected to have both symptoms on a chance basis, by means of the formula

$$\text{Expected \% (with both)} = \frac{\text{\% (J. spot)} \times \text{\% (b'down)}}{100},$$

the analysis of variance showed that significantly* more fruit had joint symptoms than expected.

There was therefore evidence of an interaction between Jonathan spot and breakdown. More fruit had both disorders than could be expected on a chance basis, which suggested that fruit in a certain physiological state (e.g. more advanced

* Analysis of variance shown hereunder:

Source	D.F.	Sum of Squares	Mean Square	*F*	Signif.
Mean deviation of actual	1	198·15	198·15	15·67	$P<0·001$
from expected	29	366·72	12·645		

maturity) was more susceptible to both; and the negative correlation of the two disorders between trees suggested that one disorder tended to supplant the other.

This interaction could have been a reason for the lack of significance of the correlation between mean fruit weight and Jonathan spot in 1952. (In 1955 the generally low level of incidence was probably the main reason.) In 1957 and 1958, when Jonathan spot was the only disorder, the correlation was highly significant (see Table 6).

TABLE 3

REGRESSIONS OF MEAN WEIGHT OF SIZE GROUP, PERCENTAGE JONATHAN SPOT, AND MEAN SEED NUMBER—WITHIN TREES: 1957 AND 1958

Mean Fruit Wt. (g)		Jonathan Spot (%)		Mean Seed No.	
1957	1958	1957	1958	1957	1958
60	60	27·6	37·2	4·31	4·79
86	86	40·0	58·0	4·80	5·84
112	112	48·4	82·3	5·35	6·95
138	—	59·6	—	5·89	

Regressions

Regression	1957		1958		Signif. of Difference of *b*'s, 1957 and 1958
	b	Signif.	*b*	Signif.	
Mean fruit wt. and % Jonathan spot	0·4015	$P<0·01$	0·8673	$P<0·05$	$P<0·01$
Mean fruit wt. and seed no.	0·0233	$P<0·01$	0·0415	$P=0·01$	$P<0·01$
% Jonathan spot and seed no.	0·0504	$P<0·05$	0·0479	$P<0·05$	$P<0·05$

(*b*) *Relation of Jonathan Spot to Seed* Content*

(i) *Within-tree Relationships*

(1) The interrelation of fruit size, Jonathan spot, and seed number was tested by an intercorrelation of the data of the size groups, which were combined when it was found that there was no evidence of variation between trees in the slope of the regressions of mean fruit weight, percentage Jonathan spot, and seed number within trees in one year (see Table 3).

* In this paper "seed number" refers to seeds of full size containing fully developed embryos. However, the number of seeds of full length but without embryos was very small and would not have affected the relationships.

(2) The mean seed number of 50 sound and 50 affected fruits of two weight classes (70–80 g and 80–90 g) from the one tree were compared for 36 trees in 1958. The results are given in Table 4. In all cases but one there was a lower seed number in the sound fruits than in those with Jonathan spot. In the 70–80 g class this was significantly less ($P<0\cdot05$ or less) in 23 of the 36 trees, and in the 80–90 g class 18 of the 36 were significantly lower. Collectively the differences were very highly significant.

TABLE 4

MEAN SEED NUMBER OF SOUND AND AFFECTED FRUITS

Class	Mean Seed Number		Mean	
	Sound Fruits	Affected Fruits	Difference	Significance
70–80 g	4·36	5·59	−1·23	$P<0\cdot001$
80–90 g	5·24	6·17	−0·93	$P<0\cdot001$

(3) Observations were also made on the number of germinated seeds present in 50 sound and Jonathan spot-affected fruits of the 80–90 g classes for all trees in 1958. Though there were very few seeds germinated in any one sample—a maximum of eight seeds and a mean difference of 1·03—this difference in favour of affected fruits was highly significant ($P<0\cdot001$).

(4) To test the effect on Jonathan spot of difference in size of fruits but with the same seed number and with other conditions as similar as possible, alternate branches of three trees were heavily thinned after the natural abscission in mid December. The results of examination are given in Table 5. There was no significant difference in Jonathan spot or seed number.

(ii) *Between-tree Relationships*

(1) The intercorrelation of the variables mean fruit weight per tree, percentage Jonathan spot, and mean seed number (seed number was available for 16 trees in 1957 and for 36 trees in 1958) was examined, and results are given in Table 6.

There was a highly significant correlation between mean fruit weight and Jonathan spot which remained highly significant when seed number was held constant; and also between Jonathan spot and seed number which remained highly significant when mean fruit weight was held constant. The relation between mean fruit weight and seed number was a weaker one, but it was significant in 1958 and remained so when percentage Jonathan spot was held constant.

The regression lines were parallel for the two years for all relationships but those for 1958 were displaced significantly from those of 1957.

The effects of cell thinning before fruit division and after cell division on mean fruit weight, percentage Jonathan spot, and mean seed number are shown in Table 7. Both thinning treatments affected fruit size to the same degree.

TABLE 5

MEAN FRUIT WEIGHT, PERCENTAGE JONATHAN SPOT, AND MEAN SEED NUMBER—THINNED AND UNTHINNED BRANCHES

Tree	Treatment	Mean Fruit Weight (g) (4 samples)			% Jonathan Spot (4 samples)			Mean Seed Number (4 samples)		
		Mean ± S.E.	Diff.	Signif.	Mean ± S.E.	Diff.	Signif.	Mean ± S.E.	Diff.	Signif.
1	Controls	80·1 ± 0·83	14·8	$P<0·001$	66·1 ± 3·8	3·2	N.S.	5·7 ± 0·020	0·2	N.S.
	Thinned	94·9 ± 0·85			69·3 ± 4·8			5·9 ± 0·030		
2	Controls	76·7 ± 0·57	15·7	$P<0·001$	89·6 ± 4·2	2·2	N.S.	5·3 ± 0·020	0·1	N.S.
	Thinned	94·4 ± 0·71			91·8 ± 3·9			5·4 ± 0·028		
3	Controls	93·3 ± 0·69	8·4	$P<0·01$	93·4 ± 3·6	−2·0	N.S.	6·6 ± 0·053	−0·2	N.S.
	Thinned	101·7 ± 0·75			91·4 ± 3·5			6·4 ± 0·031		

Fruit from blossoms thinned before fruit cell division had begun had a lower seed number than those thinned after fruit cell division had ceased. The most probable explanation of this is that in the former the reduced competition between fruitlets in the stage of rapid cell division permitted the retention of fruits of low

TABLE 6

MEAN FRUIT WEIGHT, PERCENTAGE JONATHAN SPOT, AND MEAN SEED NUMBER—BETWEEN-TREE RELATIONSHIPS: 1957 AND 1958

Attribute	1957		1958	
	Value	S.D.	Value	S.D.
Mean fruit weight	79·44	3·64	73·79	5·07
% Jonathan spot	33·52	110·89	52·34	16·57
Seed number	4·67	0·30	5·91	0·29
Regression	*b*	Signif.	*b*	Signif.
Mean fruit weight and % Jonathan spot	2·0639	$P<0·01$	2·3174	$P<0·001$
Mean fruit weight and seed number	0·0236	N.S.	0·0294	$P<0·01$
% Jonathan spot and seed number	0·0183	$P<0·01$	0·0141	$P<0·001$

Comparison of Regressions, 1957–58

Regression	Significance of Difference of *b*'s	Significance of Displacement
Mean fruit weight and % Jonathan spot	N.S.	$P<0·01$
Mean fruit weight and seed number	N.S.	$P<0·01$
% Jonathan spot and seed number	N.S.	$P<0·01$

Correlations	1957		1958	
	r	Signif.	*r*	Signif.
Mean fruit weight and % Jonathan spot	0·6880	$P<0·01$	0·7150	$P<0·001$
Mean fruit weight and % Jonathan spot; seed number	0·6939	$P<0·01$	0·5896	$P<0·01$
Mean fruit weight and seed number	0·2848	N.S.	0·5153	$P<0·01$
Mean fruit weight and seed number; % Jonathan spot	0·2505	N.S.	0·4861	$P<0·01$
% Jonathan spot and seed number	0·6585	$P<0·01$	0·7999	$P<0·001$
% Jonathan spot and seed number; mean fruit weight	0·6647	$P<0·01$	0·7202	$P<0·01$

seed number which would have abscissed under the stress of competition from fruitlets of higher seed number. The alternative that the treatment suppressed seed development is less likely, for the numbers of pollinated but partially developed seeds were not significantly different from each other or from the unthinned trees.

Blossom-thinned fruits had a lower incidence of Jonathan spot.

TABLE 7

MEAN FRUIT WEIGHT, PERCENTAGE JONATHAN SPOT, AND MEAN SEED NUMBER PER FRUIT—THINNING TREATMENTS: 1958

Treatment	No. of Trees	Mean Fruit Weight (g)			Percentage Jonathan Spot			Mean Seed Number per Fully Developed Fruit			Mean Seed Number per Partially Developed Fruit		
		Mean ± S.E.	Significance of Difference		Mean ± S.E.	Significance of Difference		Mean ± S.E.	Significance of Difference		Mean ± S.E.	Significance of Difference	
			A*	B*		A	B		A	B		A	B
Control	36	73·9 ± 0·141			52·8 ± 0·46			5·91 ± 0·008			0·50 ± 0·0015		
Blossom-thinned	4	97·8 ± 1·81	$P<0·001$	N.S.	54·5 ± 1·94	N.S.	$P<0·01$	5·02 ± 0·135	$P<0·001$	$P<0·01$	0·46 ± 0·016	N.S.	N.S.
November-thinned	4	95·0 ± 1·12	$P<0·001$		66·9 ± 2·66	$P<0·01$		5·89 ± 0·125	N.S.		0·60 ± 0·059	N.S.	

* A, between control and treated fruit. B, between treatments.

IV. Discussion

It has been shown (Martin 1954; Martin, Lewis and Cerny 1954) that there is a very close positive correlation between the incidence of the physiological disorders bitter pit and breakdown and the mean fruit weight per tree in any season, and also between fruit size and incidence within a tree. In addition there was an interaction of these two disorders, each tending to suppress the other. These principles have now been shown to apply to the disorder Jonathan spot. When it occurs alone there is a positive correlation between mean fruit weight and percentage Jonathan spot per tree, and between weight and incidence within trees. In addition, an interaction between Jonathan spot and breakdown has been demonstrated; there is a general negative correlation between the incidence of the two disorders but they occur on the same fruit to a greater amount than would be expected by chance. It is possible that all physiological disorders interact, and that all are closely correlated with: (i) mean fruit weight per tree (which is a measure of the differences of crop or the leaf/fruit ratio between trees in an otherwise uniform plot); (ii) fruit weight within trees (which is probably a measure of differences of initial vigour and degree of competition between fruits). Both are related to the amount of cell division and cell expansion which has occurred.

With Jonathan spot at least,* there is a further factor, namely, the seed. Fruits with this disorder have a greater mean seed number than sound fruits. Within and between trees the variables fruit weight, percentage Jonathan spot, and seed number are positively intercorrelated; and the partial correlation of Jonathan spot and seed number, holding fruit size constant, remains highly significant.

Further evidence of the seed–disorder relation was obtained from thinning experiments. Within trees, a large increase in fruit size, achieved without affecting seed number, gave no increase in Jonathan spot. Between trees, treatment which produced populations of similar size, but different seed number, gave different levels of Jonathan spot.

Seeds of fruits with Jonathan spot had a greater tendency to germinate within the fruit than those of sound fruits; but there was no evidence to disclose whether this was an attribute of the seeds themselves or alternatively that they had been influenced by products from disordered fruits.

It is apparent that there exists an important connection between seed number and susceptibility to this disorder. It is unlikely that substances produced by the seed could cause the development of the lesions in the way that Poapst and Phillips (1958) have shown for the disorder core flush. The latter develops in tissues adjacent to the carpels, whereas Jonathan spot is virtually confined to the epidermis, at a maximum distance from the seed. The vascular connection, if any, would be extremely circuitous, and direct diffusion through the flesh improbable.

It is suggested that those conditions which are associated with relatively large seed numbers influence the physiological characteristics of the fruit. Such conditions would include: a higher proportion of fully developed embryo sacs (Dorsey 1929),

* No other physiological disorders have appeared in this plot in recent years, but examination of this variety from other plots have shown that fruits with breakdown or deep scald do not have significantly different seed numbers from sound fruits of the same weight.

an increased abscission of fruitlets with lower seed number, and those favouring better pollination and fertilization. A higher number of embryos necessary to prevent abscission implies a different physiological state in the fruitlet; this could be further aggravated or initiated by the competition from the embryos during the critical stages of the cell development in the flesh. If seeds have a direct effect it is most likely to occur in the early stage of fruit development, for Abbott (1958) has shown that from the fourth to the seventh week after pollination the seeds can be removed and a simple abscission inhibitor, such as naphthaleneacetic acid, can replace them; after the seventh week, both seed and inhibitor are unnecessary.

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